

**DEVELOPMENT AND ASSESSMENT OF ANTI-DIABETIC
POTENTIAL OF DRUG ENCAPSULATED GUAR GUM
NANOFORMULATION FOR ORAL USE WITH SUSTAINED
RELEASE**

Thesis Submitted for the Award of the Degree of

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DECLARATION

I, hereby declared that the presented work in the thesis entitled “**Development and Assessment of Anti-Diabetic Potential of Drug Encapsulated Guar gum Nanoformulation for oral use with sustained release**” in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of **Dr. Jeena Gupta**, working as Associate Professor, in the Department of Biochemistry, School of Bioengineering and Biosciences of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigators. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled **“Development and Assessment of Anti-Diabetic Potential of Drug Encapsulated Guar gum Nanoformulation for oral use with sustained release”** submitted in fulfillment of the requirement for the award of degree of Doctor of Philosophy (Ph.D.) in the Department of Biochemistry, School of Bioengineering and Biosciences, is a research work carried out by Sourbh Suren Garg, 12014510, is bonafide record of his original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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Abstract

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or a combination thereof. The global incidence of type 2 diabetes continues to rise, driven by demographic shifts, lifestyle changes, and increased obesity prevalence. This escalating burden necessitates improved therapeutic strategies targeting effective glycemic control to prevent or delay diabetic complications including nephropathy, neuropathy, retinopathy, and cardiovascular disease. Despite numerous antidiabetic agents, limitations such as low bioavailability, adverse effects, and poor patient adherence restrict their clinical utility, emphasizing the critical need for advanced drug delivery platforms that can enhance pharmacokinetic and pharmacodynamic properties.

Acarbose, a clinically approved α -glucosidase inhibitor for type 2 diabetes management, mitigates postprandial hyperglycemia by delaying carbohydrate digestion and absorption. However, its therapeutic efficacy is impeded by low oral bioavailability, limited gastrointestinal absorption, and dose-dependent gastrointestinal adverse effects necessitating frequent administration. To address these challenges, nanodelivery systems incorporating natural polysaccharides such as guar gum have been investigated for their biocompatibility, and ability to improve drug release and stability. The gel-forming ability of guar gum can modulate drug release kinetics and extend gastrointestinal residence time, thereby potentially enhancing the systemic availability and therapeutic efficacy of hydrophilic agents such as acarbose.

This study reports the development and characterization of acarbose-encapsulated guar gum nanoformulation (AEGN), designed to achieve sustained drug release, reduce acarbose-associated side effects, and enhance therapeutic efficacy. The solid dispersion approach facilitated molecular dispersion of acarbose within the polysaccharide matrix, promoting amorphization and enhanced drug release. Particle size analysis confirmed the formation of nanoparticles with uniform distribution, while zeta potential measurements indicated a negative surface charge, supporting colloidal stability. Fourier-transform infrared spectroscopy (FTIR) and powder X-ray diffraction (pXRD) analyses confirmed molecular interactions between acarbose and guar gum, along with the conversion of crystalline acarbose into an amorphous state, both contributing to enhanced release behaviour. Scanning electron microscopy (SEM) of the spray-dried formulation revealed mostly spherical particles with smooth surfaces, suggesting uniform morphology and efficient solid dispersion of acarbose

within the guar gum matrix. *In vitro* drug release studies indicated sustained acarbose release from AEGN formulations, contrasting the rapid release observed with free acarbose. This release profile aligns with the hydrophilic matrix nature of guar gum, which slows drug diffusion and prolongs gastrointestinal residence time, thereby supporting sustained pharmacological action and potentially reducing the required dosing frequency. The sustained release is expected to contribute to attenuated postprandial glucose excursions and minimized gastrointestinal side effects.

AEGN formulations demonstrated significantly enhanced antioxidant capacity, as evidenced by improved free radical scavenging activities compared to native acarbose and guar gum. This enhancement likely results from the combined effects of the acarbose and intrinsic antioxidant properties of guar gum. Enzymatic inhibition assays revealed superior α -amylase and α -glucosidase inhibitory activity of the nanoformulations relative to free drug, likely due to improved drug dispersion and sustained release from the guar gum matrix, which promotes more effective interaction with carbohydrate-digesting enzymes. Cytotoxicity evaluations using MTT assays in cells cultured under both normoglycemic and hyperglycemic conditions demonstrated that AEGN formulations maintained high cellular viability across tested concentrations. Additionally, lactate dehydrogenase (LDH) release assays showed reduced membrane damage following AEGN treatment, indicating protective effects. Moreover, the formulation increased antioxidant enzyme levels and mitigated oxidative stress in cells exposed to hyperglycemic conditions. Collectively, these results indicate that AEGN enhances the antioxidant defense system under hyperglycemic stress while maintaining cellular integrity. Among the formulations tested, AEGN-3 demonstrated superior efficacy and was therefore selected for subsequent *in vivo* pharmacological evaluation.

The pharmacodynamic efficacy of AEGN-3 was evaluated in a high-fat diet (HFD)-fed, streptozotocin (STZ)-induced diabetic rat model. Oral administration of AEGN-3 significantly reduced fasting blood glucose levels and body weight compared to untreated diabetic controls and animals treated with native acarbose and guar gum. Serum analysis demonstrated improvements in hepatic and renal function markers, as well as lipid metabolism following AEGN-3 treatment. Histopathological examination of hepatic, renal, pancreatic, and cardiac tissues revealed restored architecture and reduced inflammation, indicating protective and regenerative effects. Additionally, biochemical assays showed restoration of superoxide dismutase (SOD), catalase, and glutathione-s-transferase (GST), alongside a significant decrease in lipid peroxidation (LPO) biomarkers, acetylcholinesterase (AChE) and xanthine

oxidase activities, confirming effective mitigation of oxidative stress and inflammatory markers, particularly tumor necrosis factor- α (TNF- α) and interleukins (IL). Integrative network pharmacology analysis was employed to elucidate the molecular mechanisms underpinning the observed pharmacological effects. The analysis identified key signaling pathways modulated by the acarbose, including PI3K/Akt, AMPK, insulin receptor, and inflammatory pathways, which collectively regulate glucose metabolism, oxidative stress responses, and inflammatory cascades. These findings align with the action of acarbose in improving glycemic control and reducing oxidative damage, supporting the enhanced therapeutic potential observed experimentally.

In conclusion, the guar gum-based solid dispersion nanoformulation markedly improves physicochemical characteristics, antioxidant capacity, enzymatic inhibitory activity, biocompatibility, and *in vivo* antidiabetic efficacy of acarbose. Its sustained release profile, coupled with synergistic antioxidant effects and protection against hyperglycemia-induced cellular damage, establishes a robust platform for enhanced oral antidiabetic therapy. Mechanistic insights derived from network pharmacology further validate the multifunctional therapeutic benefits enabled by this natural polymeric delivery system. These results highlight the potential of polymer-based nanocarriers to address the limitations of conventional hydrophilic antidiabetic agents, paving the way for the development of next-generation oral formulations with superior efficacy and safety. Further translational research, including clinical trials and scale-up manufacturing is essential to validate and advance the clinical application of this strategy for comprehensive diabetes management.

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ॐ त्र्यम्बकं यजामहे सुगन्धिं पुष्टिवर्धनम्।
उर्वारुकमिव बन्धनान् मृत्योर्मुक्षीय मामृतात्॥

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Mahadev, the silent ascetic and compassionate protector, has been my unseen guide through moments of doubt, exhaustion, and uncertainty. His divine presence has helped me rise above every challenge on this journey.

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Abbreviations

Full Term	Abbreviation
Bovine Serum Albumin	BSA
Reactive Oxygen Species	ROS
Streptozotocin	STZ
High-Fat Diet	HFD
Normal Pellet Diet	NPD
Advanced Glycation End Products	AGEs
Nuclear Factor-kappa B	NF- κ B
Interleukin	IL
Tumor Necrosis Factor- α	TNF- α
Adenosine Triphosphate	ATP
Superoxide Dismutase	SOD
Glutathione-S-Transferase	GST
Lactate Dehydrogenase	LDH
Vascular Endothelial Growth Factor	VEGF
Matrix Metalloproteinase	MMP
Polyvinylpyrrolidone	PVP
Acarbose-encapsulated guar gum nanoformulation	AEGN
Acetylcholinesterase	AChE
Malondialdehyde	MDA
Serum Glutamic-Oxaloacetic Transaminase	SGOT
Serum Glutamic-Pyruvic Transaminase	SGPT
Alkaline Phosphatase	ALP
Gamma-Glutamyl Transferase	GGT

Very low-density lipoprotein	VLDL
Low-density lipoprotein	LDL
High-density lipoprotein	HDL
Hydrogen peroxide	H ₂ O ₂
Protein-protein interaction	PPI
Gene ontology	GO
Kyoto Encyclopedia of Genes and Genome	KEGG
Root Mean Square Deviation	RMSD
Root Mean Square Fluctuation	RMSF
2,2-diphenyl-1-picrylhydrazyl	DPPH
2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)	ABTS
Lipid peroxidation	LPO

Chapter 1

Introduction

Obesity, diabetes, and cardiovascular disorders are common lifestyle disorders that occur as a result of changes in food eating, behaviour, and lack of regular exercise (Li et al., 2020). Amongst these disorders, diabetes is a major public health challenge that impacts the world population on a great scale (Mathur et al., 2022). The historical background of diabetes traces back to ancient times, with the disease first documented in the Ebers Papyrus around 1550 BC, where frequent urination was described as a major symptom (Soczewka et al., 2023). The term “diabetes” was introduced by the Greek physician Aretaeus in the 2nd century AD, meaning “to pass through,” and later refined as “diabetes mellitus” in the 17th century to distinguish the condition by its sweet-tasting urine (Karamanou et al., 2016). Over centuries, diabetes has evolved from being a rare disease to a major global health challenge, particularly in low- and middle-income countries. India, often referred to as the diabetes capital of the world, contributes significantly to the global burden of the disease. The weighted prevalence of diabetes in Haryana was higher than the national average (12.4% vs. 11.4%). The prevalence was higher in urban (17.9%) than in rural areas (9.5%). The prevalence of diabetes in rural areas was higher in the NCR region, while that of prediabetes was higher in rural non-NCR region (Kalra et al., 2024).

The major hallmark of diabetes mellitus is hyperglycemia which is characterized by defects in insulin secretion or by the failure of the pancreas to produce insulin (Daryabor et al., 2020). According to the IDF Diabetes Atlas 2025, an estimated 589 million adults (20-79 years) are currently living with diabetes worldwide. This number is projected to increase to 853 million by 2050, affecting approximately 1 in 8 adults globally (International Diabetes Federation, 2025). Compared to women, diabetes mellitus mainly affects men with higher incidence, and it is more prevalent in low- and middle-income countries (International Diabetes Federation, 2021). Worldwide India, China, and the United States are the leading countries with the highest prevalence of diabetes and the highest incidence of diabetes-associated mortality (Bragg et al., 2022). The continuous increase in patients with diabetes will further raise the global diabetes mortality burden, which accounted for approximately 3.4 million deaths in 2024. This trend is expected to significantly increase both the economic and health burdens worldwide, with the current global cost estimated at USD 1.015 trillion, projected to rise substantially by 2030 to 1.043 trillion (International Diabetes Federation, 2025).

Several reports have confirmed that under hyperglycemic conditions, there is an increased ability/transition of glucose to get converted into enol which is called glucose autooxidation (Giri et al., 2018; Garg et al., 2022). This reaction serves as a substantial source of free radicals such as hydrogen peroxide (H_2O_2) and superoxide anion which ultimately cause oxidative damage to protein, cell membrane, and DNA (Nishikawa et al., 2000). Furthermore, free radical generation was found to decrease the activity of antioxidant defense enzymes such as catalase, SOD, and reduced glutathione (Rajput et al., 2021). Since diabetes and diabetes-induced oxidative stress are closely linked, thus intruding this link is of utmost importance to reduce the mortality index worldwide. For instance, eating a nutritious diet and exercising regularly are the first-line treatments for effective management of diabetes. Several theories highlighted that regular exercise improves blood glucose control in diabetics and prevents the occurrence of other ailments such as cardiovascular risk (Zhong et al., 2024; Klesges et al., 1992). However, if diet and lifestyle changes are unable to control diabetes, diabetics are advised to start anti-diabetic therapies (Asif, 2014; Jacob & Narendhirakannan, 2019). There are many synthetic anti-diabetic drugs available in the global market for the treatment of diabetes, and most of them have been reported to possess side effects (Patel et al., 2012). For instance, sulfonylureas, biguanides, and glinides are some oral hypoglycemic drugs that are used to lower glucose levels. Sulfonylurea is a hypoglycemic agent that works as an anti-diabetic agent by lowering glucose levels. Briefly, this hypoglycemic agent closes the adenosine triphosphate (ATP)-sensitive potassium channels by acting on the pancreatic β -cell membrane, which further secretes the insulin. A recent study by Mehrpour et al. reported that dizziness, tremor, diaphoresis, and agitation are the major side effects associated with sulfonylurea therapy (Mehrpour et al., 2022). It has been found that elevated levels of cytochrome b5 reductase 3 enhance lifespan in mice by improving metabolic parameters (Martin-Montalvo et al., 2016). However, a recent study by Watanabe et al. showed that sulfonylurea treatment impairs cytochrome b5 reductase 3 levels and decreases islet glucose utilization (Watanabe et al., 2023). Focusing on this aspect, Fan et al. showed that ablation of cytochrome b5 reductase 3 (Cyb5r3) reduced insulin release and cause glucose intolerance in FoxO1-deficient β -cells (Fan et al., 2020). Similarly, Watanabe et al. showed that supplementation of sulfonylurea in the *in vivo* and *ex vivo* murine islets reduced glucose consumption in pancreatic β -cells followed by a reduction in cytochrome b5 reductase 3 frequency (Watanabe et al., 2023). Together these studies conclude that chronic exposure to sulfonylurea affects insulin secretion in diabetes.

Another oral medication used for the treatment of hyperglycemia belongs to the class of Biguanide called Metformin. This anti-diabetic drug inhibits gluconeogenesis in the liver and reduces blood glucose levels in the body; however, nausea, diarrhoea, and abdominal discomfort were reported after receiving metformin therapy (Sola et al., 2015). While metformin and sulfonylureas are effective, their side effects and limited mechanisms of action highlight the need to explore other anti-diabetic drugs. In this context, acarbose, an α -glucosidase inhibitor, is widely used to manage postprandial hyperglycemia. Recent evidence suggests that acarbose can also influence gut microbiota composition. Studies report that acarbose increases the abundance of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, while reducing certain pathogenic species. These alterations in gut microbiota may improve glucose metabolism, enhance insulin sensitivity, and reduce systemic inflammation, highlighting an additional mechanism by which acarbose can contribute to diabetes management. However, conventional acarbose therapy is limited by rapid absorption, short half-life, frequent dosing requirements, and gastrointestinal side effects such as flatulence and diarrhoea. Its immediate-release profile provides only transient postprandial glucose control, which may not be sufficient for sustained therapeutic outcomes. Understanding these interactions is important for optimizing acarbose therapy. It also helps predict potential metabolic benefits beyond glycemic control.

To overcome these side effects, phytocompound-based therapies are increasingly explored for diabetes management, as they contain bioactive molecules capable of reducing hyperglycemia and hyperglycemia-induced oxidative stress with minimal side effects. However, these bioactive components also present several challenges, including poor solubility, short half-life, and low oral bioavailability (Tran et al., 2020; Rahman et al., 2022). These drawbacks together reduce the therapeutic efficacy of anti-diabetic therapies which suggests that there is an imperative need to look for an alternative that would improve the therapeutic efficacy by improving solubility, half-time, and bioavailability.

Polymers impersonate an influential role in the successful delivery of a drug (Sabbagh et al., 2022). Polysaccharides are the universal polymers obtained from nature. The polysaccharides have been broadly used because they manifest high bioavailability, are easily available, biodegradable, and environmentally friendly. More importantly, they are non-toxic so that they do not cause any damage to the tissue or organ and do not interfere with the functioning of any organ. Guar gum is a naturally occurring polymer that does not corrode easily, which makes it proficient for effective drug delivery in targeted area. In addition to its pharmaceutical

applications, guar gum cultivation is primarily concentrated in the arid and semi-arid regions of India (Mahdipour-Afra et al., 2021), where farmers rely on traditional rain-fed agricultural practices with minimal irrigation and chemical inputs. This makes guar gum a highly sustainable crop that not only supports rural livelihoods but also holds global economic importance due to its rising demand in food, pharmaceutical, and oil industries (Mudgil et al., 2014). The stomach has a highly acidic pH, which can degrade orally administered drugs, and microorganisms in the small intestine may further compromise drug stability (Liu et al., 2017). In addition to the acidic effect, microorganisms present in the small intestine also spoil the drug (Wahlgren et al., 2019). Polymers such as guar gum are therefore employed to enhance drug stability in the gastrointestinal tract. To date, significant progress has been made using guar gum to improve drug solubility and bioavailability. However, conventional acarbose formulations, despite their efficacy, face limitations including rapid absorption, short half-life, frequent dosing, and gastrointestinal side effects. To overcome these challenges, this study develops a guar gum-based nanoformulation of acarbose to provide sustained drug release, enhance therapeutic efficacy, improve oral bioavailability, reduce dosing frequency, and minimize side effects, effectively addressing the shortcomings of current therapy.

Chapter 2

Review of Literature

This chapter provides a comprehensive overview of the existing literature on the pathophysiology, management strategies, and recent advancements in the treatment of diabetes mellitus. It contextualizes the significance of the present study by summarizing key developments in the field, identifying research gaps, and outlining the rationale for the selected approach. The chapter discusses diabetes, its types, complications, currently available therapies, and emerging drug delivery strategies, including nanoformulations. The need for improved drug delivery is explored with a focus on natural polymers, particularly guar gum, as a promising carrier for controlled and targeted delivery. The development of such formulations is aimed at improving drug solubility and pharmacokinetics, enhancing therapeutic outcomes, minimizing the side effects associated with conventional antidiabetic treatments, and supporting the advancement of optimized formulations toward clinical evaluation and regulatory approval.

2.1 Introduction to diabetes

Diabetes stands out as a significant global public health concern, affecting a substantial portion of the world's population and posing serious health challenges. The primary characteristic of diabetes mellitus is elevated blood sugar levels, resulting from either inadequate insulin secretion or the inability of the pancreas to produce insulin (Garg & Gupta, 2022). Insulin is a vital hormone that exerts its function by regulating blood sugar levels and facilitating the entry of glucose into cells for energy production. However, when insulin function is compromised, this mechanism is disrupted, causing glucose to accumulate in the bloodstream, leading to a striking rise in blood sugar levels that defines diabetes (Petersen & Shulman, 2018).

In general, diabetes is classified into Type 1 diabetes and type 2 diabetes, gestational diabetes, and prediabetes. Among these, type 1 diabetes and type 2 diabetes are the most common forms, affecting a large portion of population, with consequences often linked to sedentary lifestyles and lack of physical activity (Antar et al., 2023). Type 1 diabetes is most commonly diagnosed in childhood or early adulthood and results from an autoimmune destruction of insulin-producing cells in the pancreas. This compromises the pancreas's ability to produce insulin, impairing glucose entry into cells. In contrast, type 2 diabetes, the most prevalent form, typically affects adults and is characterized by insulin resistance, where cells fail to respond properly to insulin despite sufficient production by the pancreas. This results in elevated blood

glucose levels (Krause & De Vito, 2023). Gestational diabetes, another form of diabetes, occurs during pregnancy and usually resolves postpartum but increases the risk of developing type 2 diabetes later. Additionally, prediabetes refers to blood sugar levels higher than normal but not high enough for a diabetes diagnosis, often serving as a precursor to type 2 diabetes (Nakshine & Jogdand, 2023).

2.2 The escalating worldwide burden of diabetes

Diabetes has reached pandemic levels and is now recognized as a global public health challenge, with countries like India, China, and the United States having the highest numbers of people affected (Garg & Gupta, 2022). In India and China, factors such as urbanization, sedentary lifestyles, and obesity have significantly increased the prevalence of type 2 diabetes (International Diabetes Federation, 2025).

According to the IDF Diabetes Atlas, 11th Edition (2025), an estimated 589 million adults aged 20-79 were living with diabetes in 2024, about 11.1% of the global adult population. This number is projected to rise to 853 million by 2050. Type 2 diabetes constitutes over 90% of all diagnosed cases. Alarmingly, around 252 million adults have undiagnosed diabetes, with 158 million over the age of 65. Additionally, 635 million adults show impaired glucose tolerance, increasing their risk for developing diabetes. In 2024, global diabetes care costs exceeded USD 1 trillion, representing approximately 12% of total health expenditure. Diabetes was responsible for over 3.4 million deaths, or 9.3% of all global deaths (International Diabetes Federation, 2025).

Regionally, the Western Pacific has the largest number of adults with diabetes (215 million), accounting for approximately one in every eight people. The Middle East and North Africa region has the highest prevalence, with one in six adults affected. The African region is projected to experience the greatest relative increase, with the number of people with diabetes expected to rise by 142%, reaching 60 million by 2050. Europe has the highest number of people with type 1 diabetes (2.7 million), while North America and the Caribbean account for the highest healthcare spending (USD 439 billion, 43% of global costs). In South and Central America, nearly one in three adults with diabetes is undiagnosed. In South-East Asia, one in three live births is impacted by hyperglycaemia during pregnancy, the highest among all regions.

Diabetes is associated with severe complications. People with type 2 diabetes have a 72% higher risk of heart attack, 52% higher risk of stroke, 84% higher risk of heart failure, and a

56% increased risk of developing dementia, especially if diabetes starts early. Nearly 25% of adults with diabetes show signs of diabetic retinopathy, with over 10% at risk of vision loss. Furthermore, 9.1 million people are estimated to be living with type 1 diabetes, mostly aged 20-59, and around 23 million live births are affected by hyperglycaemia during pregnancy annually (International Diabetes Federation, 2025).

2.3 The multifaceted complications of diabetes

A wide range of complications is associated with diabetes, particularly type 2 diabetes, where persistent hyperglycemia can impair organ function and exacerbate disease complications. Among the various complications linked to type 2 diabetes, the most critical include diabetic nephropathy, diabetic neuropathy, delayed wound healing, and diabetic retinopathy.

2.3.1 Diabetic nephropathy

The kidneys are vital organs responsible for filtering waste, maintaining fluid and electrolyte balance, and removing toxins from the blood. Each kidney contains millions of nephrons, comprising two main components: the glomerulus and renal tubules. The glomerulus, a capillary network at the start of the nephron, is the primary site of blood filtration (Garreta et al., 2022). High pressure forces water, ions, glucose, amino acids, and waste products like creatinine and urea into the Bowman's capsule, forming glomerular filtrate. This filtrate then passes through the renal tubules, where essential substances are reabsorbed and waste is excreted via urine (Thompson & Joy, 2022). The urine flows through the collecting ducts to the renal pelvis and bladder, maintaining homeostasis. The renin-angiotensin-aldosterone system (RAAS) regulates kidney function by maintaining blood pressure and fluid balance. In response to low blood pressure or sodium levels, the kidneys release renin, which converts angiotensinogen to angiotensin I and then to angiotensin II (Ma et al., 2022; Lin et al., 2018). Angiotensin II promotes aldosterone and antidiuretic hormone (ADH) release, aiding water retention, vasoconstriction, and blood pressure elevation. These mechanisms ensure proper kidney function but are disrupted under hyperglycemic conditions, contributing to diabetic nephropathy (Patel et al., 2017).

Chronic hyperglycemia induces structural and functional kidney changes. Elevated glucose levels promote oxidative stress via advanced glycation end products (AGEs), damaging the glomerular filtration barrier and impairing the kidney's ability to remove creatinine and urea (Khalid et al., 2022; Wu et al., 2023). This damage leads to protein leakage, causing microalbuminuria and proteinuria. Simultaneously, hyperglycemia activates RAAS, raising

intraglomerular pressure and causing hyperfiltration. This results in glomerular basement membrane thickening, mesangial expansion, podocyte loss, inflammation, and renal fibrosis (Wang et al., 2022). As nephrons lose filtering capacity, waste accumulation and fluid imbalance occur, progressing to chronic kidney disease and potentially end-stage renal disease (Kumar et al., 2023), as mentioned in Figure 2.1.

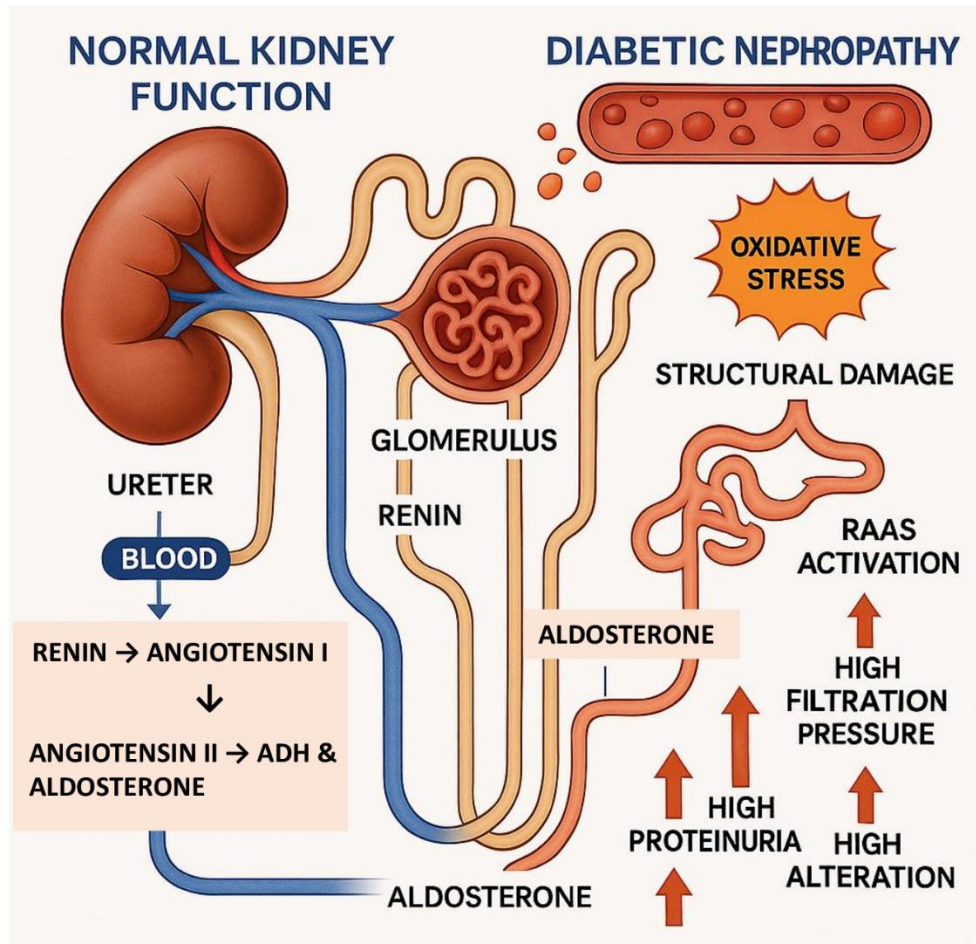


Figure 2.1: Schematic representation illustrating normal renal physiology and the pathological mechanisms involved in diabetic nephropathy.

2.3.2 Diabetic neuropathy

The transmission of signals between the brain, spinal cord, and other body parts is mediated by nerves, which are primarily responsible for sensation, movement, and the proper functioning of body organs. To maintain nerve health and proper function, blood vessels provide a sufficient supply of oxygen and essential nutrients, such as glucose, to produce energy for nerve cells. However, any abnormality in these nerves, such as prolonged exposure to high blood sugar, can impair their ability to transmit signals, leading to the development of diabetic neuropathy (Roh et al., 2016). Under hyperglycemic conditions, excess glucose begins to accumulate in

tissues and causes nerve damage. This condition can be reversed when blood glucose levels are stable. Several mechanisms are chiefly responsible for this damage, one of which is hyperglycemia and the accumulation of AGEs. In this process, glucose in excess in the bloodstream binds with macromolecules, leading to the formation of AGEs, which accumulate in various tissues of the body, including nerves. This accumulation causes structural modifications in proteins, leading to stiffening of blood vessels, which in turn causes endothelial dysfunction, impairing nerve function. Furthermore, prolonged high glucose levels impair blood flow to nerve tissues, depriving them of oxygen and essential nutrients, contributing further to disease development (Khalid et al., 2022). Another critical factor in diabetic neuropathy is oxidative stress, in which high glucose levels disrupt the balance between antioxidants and oxidants, causing oxidative stress that subsequently damages nerve function (Pang et al., 2020). Some other biochemical pathways, such as the polyol pathway, also play a crucial role in the progression of diabetic neuropathy.

2.3.2.1 The role of Polyol pathway

It is a two-step reaction mechanism, in which the first step involves the conversion of glucose to sorbitol with the action of the aldose reductase enzyme. In contrast, another enzyme called sorbitol dehydrogenase mediates the conversion of sorbitol to fructose with the production of NADH from NAD⁺ (Li et al., 2008). Therefore, the pathway is considered to be an important metabolic pathway that has determined the development of diabetes by altering the redox balance between NAD⁺ and NADH. This pathway is responsible for about 30% of glucose flux in the body and is considered one of the major pathways that play an important role in diabetes mellitus by counterbalancing the redox state. Aldose reductase and sorbitol dehydrogenase are two enzymes that catalyse the reaction and alter the redox equilibrium through the polyol pathway (Chung et al., 2003). Because of these enzymes, the polyol pathway is also called the sorbitol-aldose reductase pathway. Oxidative stress develops when there is an increase in the level of reactive oxygen species (ROS) and a reduction in the level of antioxidants in the body (Pizzino et al., 2017). It is well known that continuous oxidation and reduction reactions (also called redox reactions) occur in the body for the purpose to maintain the redox environment (Sarsour et al., 2009). But when this environment is unbalanced, the ROS causes oxidative damage to DNA, RNA, and lipids often referred to as oxidative stress. Oxidative stress has been reported to not only enhance their oxidation but also to activate various factors including nuclear Factor-kappa B (NF- κ B) and protein kinase C (Mohamed et al., 1999). The generation of reactive oxygen species (ROS), non-enzymatic glycation, activation of the polyol pathway,

and auto-oxidation of glucose are major contributors to oxidative stress in diabetes (Nishikawa et al., 2000), as depicted in Figure 2.2.

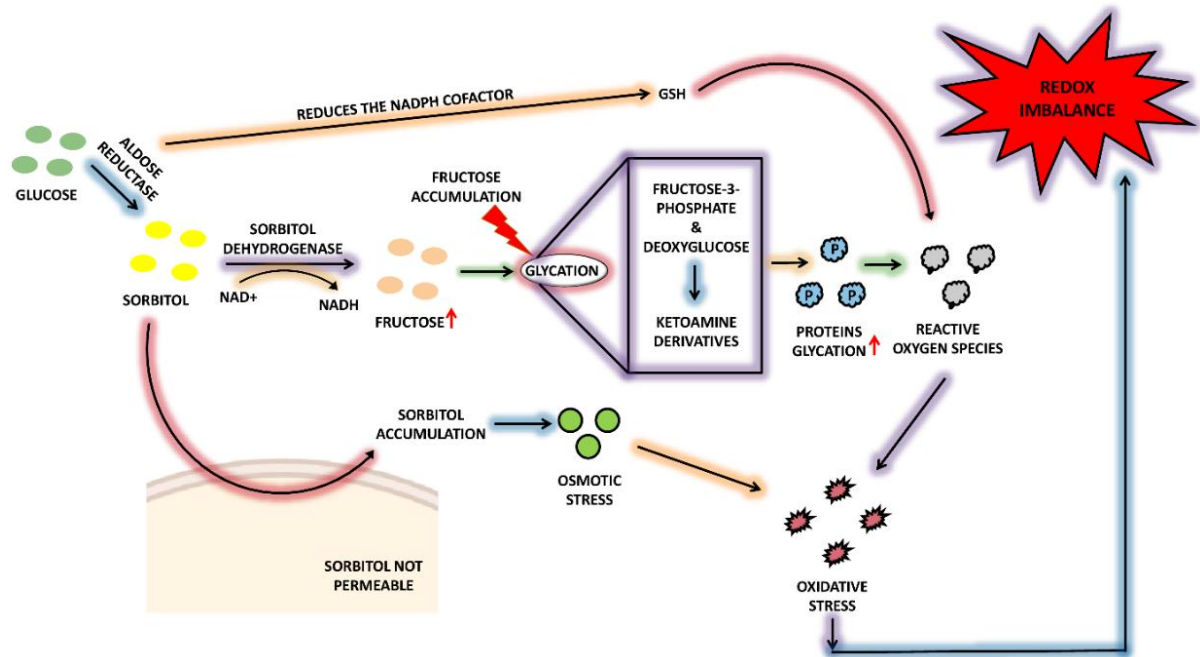


Figure 2.2: The underlying mechanism of the enzyme involved in the polyol pathway to perturbed redox balance.

2.3.4 Delayed wound healing

Delayed wound healing is a complex phenomenal defect that prevents the skin to regain its original shape, anatomical and morphological characteristics. The skin is an excellent barrier against microbial pathogens while chronic wounds are the active sites for microbiome to propagate by weakening the immune system (Bowler et al., 2001). A total of four steps is mainly involved in the healing of wounds including hemostasis, inflammation, proliferation, and remodeling (Figure 2.3). These steps equally help to restore the body's anatomical and morphological characteristics.

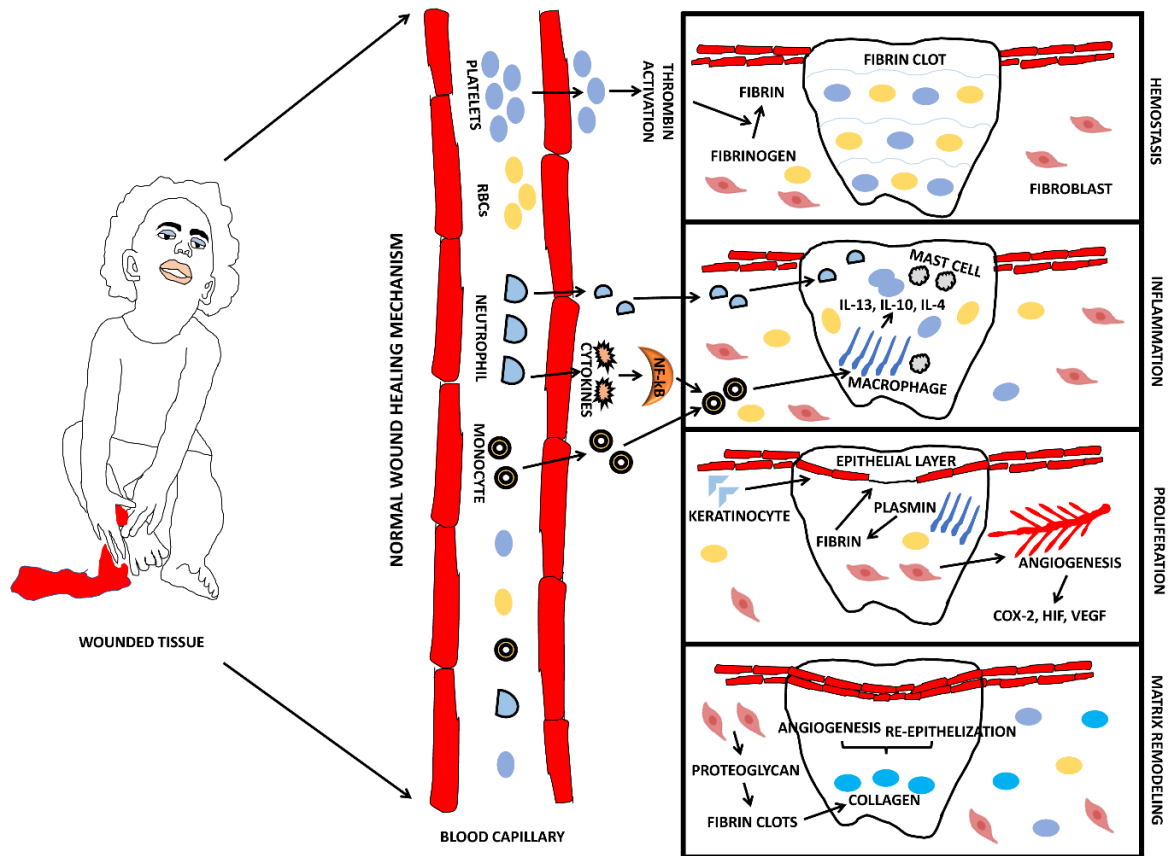


Figure 2.3: Schematic representation of the wound healing process involving platelet activation, neutrophil and macrophage recruitment, angiogenesis, matrix deposition, and collagen-mediated tissue remodeling.

2.3.4.1 Hemostasis

Excessive blood flow through the blood vessels after injury results in blood loss. However, damaged blood vessels at the wound site begin to constrict and activate the blood-clotting pathway. This cascade prevents the loss of blood thus maintaining the hemostasis of the body (Velnar et al., 2009). Platelets are activated after encountering with vascular subendothelial matrix and play an important role during blood vessel damage. Glycoprotein VI is a well-known platelet receptor with the goal to interact with various proteins present in the extracellular matrix. These glycoproteins include Von Willebrand factor, fibronectin, and collagen that support platelet attachment to the damaged blood vessel wall (Golebiewska & Poole, 2015). Thrombin, an enzyme acts as a bipolar molecule having procoagulant and anticoagulant properties and is responsible for activating platelets. Thrombin exists in its zymogen form called prothrombin; an inactive enzyme presents in the liver. Thrombin in its active state converts fibrinogen (an inactive form of fibrin) to fibrin (an active form of

fibrinogen) and is thus involved in blood clotting (Weisel & Litvinov, 2017). Three components factor V, thrombin, and prostacyclin together prevent the occurrence of platelet aggregation. Additionally, endothelial and smooth muscle cells contribute to their role by releasing platelet-derived growth factors and helping to repair the damaged vessel wall (Kingsley et al., 2002).

2.3.4.2 Inflammation

During any injury or pathogenic infection, the immune system signals the migrating cells and damaged tissue to release leukotrienes, prostaglandins, and histamines, resulting in the inflammation of injured tissue. Neutrophils, monocytes, and macrophages are immune cells that play an important role during injury or infection. In wounds, the release of histamine is initiated by mast cells whose main function is to assist in the recruitment of neutrophils to the inflammation site (Dong et al., 2020). Among all immune cells, neutrophils are the only immune cells that reach the site of infection or injury quickly. Damaged vessels are the major source of neutrophils in the injured area. During injury, various inflammatory pathways are regulated and participate in the process. During this, neutrophils at the injury site begin to secrete cytokines that mediate the activation of NF- κ B (Kolaczowska & Kubes, 2013). Activation of inflammatory pathways by NF- κ B and IL results in uncontrolled inflammation which ultimately delays the healing of an injury. In this context, circulating monocytes enter the injured site and begin to differentiate themselves into macrophages. Macrophages are highly specialized immune cells, which can engulf pathogens (also called engulfing bodies) and, are thus mainly involved in the healing of damaged tissues. The lipopolysaccharides (LPS) present on the outer wall of Gram-negative bacteria act as a potent activator of macrophages. A cascade of signal transduction is activated when macrophages are exposed to bacterial LPS which results in increased ROS, growth factors such as vascular epidermal growth factor, PDGF, and inflammatory cytokines such as TNF- α and IL-6 and thus increasing inflammation (Delavary et al., 2011). Activated macrophages also accelerate the production of pro-inflammatory cytokines such as IL-13, IL-10, and IL-4 at the wound site. Recent literature had also shown that circulating T-cells have the ability to resolve uncontrolled inflammation in wounds (Campbell et al., 2013).

2.3.4.3 Proliferation

In this phase, fibroblasts, keratinocytes, macrophages, and endothelial cells are activated which are responsible for the deposition of matrix, initiation of angiogenesis, and closure of wounds (Wilkinson & Hardman, 2020). It has been studied that, any kind of variation in electrical

gradients or mechanical tension results in the activation of keratinocytes. In addition, exposure to cytokines, hydrogen peroxide, and growth factors supports the activation of keratinocytes in the proliferative phase of wound healing. As a result of this activation, keratinocytes mediate epithelial-mesenchymal transitions and initiate re-epithelization in wounds (Landén et al., 2016). Keratinocytes support the dissociation of the integrin receptor which requires matrix metalloproteinases (MMP), particularly (MMP-1 and MMP-9) for them to migrate properly. Plasmin is a protease that degrades the fibrin-containing wound and thus helps in the movement of keratinocytes. As the movement of keratinocytes ceases, a thin layer formed by the epithelial cells (epithelial layer) is repaired (Szabo, & Hunyadi, 2004). In addition to the epithelial layer, they also reform into the basement layer and eventually regenerate the epidermis followed by terminal differentiation. Another type of cell called fibroblast cell replaces the matrix with granulation tissue that is rich in proteoglycan, fibronectin, or immature collagen. This granulation tissue subsequently supports the deposition of the mature extracellular matrix and angiogenesis and thus signals the fibroblasts to migrate and differentiate (Olczyk et al., 2014). These changes prompt endothelial cells to grow or fuse with new blood vessels in the wound area after proliferation to strengthen their network. During angiogenesis, macrophage governs the remodeling of new vasculature and generates MMP, which mediates the migration of endothelial cells (Poché et al., 2015).

2.3.4.4 Matrix remodeling

In this phase of wound healing, fibroblast cell uses fibronectin, hyaluronan, and proteoglycans to replace fibrin clots. With this removal, remodeling of the extracellular matrix begins at the injured site and results in the formation of mature collagen fibrils that help in wound repair at later stages (Darby et al., 2014). Proteoglycans help in the migration of cells through the formation of mature and cross-linked collagen fibrils. In wound healing, type III collagen is replaced by type II collagen which helps in increasing the tensile strength for scar formation (Mathew-Steiner et al., 2021). Collagen mediates the growth of new tissues and initiates the process of angiogenesis and re-epithelization by attracting fibroblast cells. In the extracellular matrix, collagen inactivates MMPs which helps in the faster healing of wounds (Duca et al., 2004).

As mentioned earlier, all these phases contribute to the rapid healing of wounds in non-diabetic individuals; however, under diabetic conditions, these phases are altered. Chronic hyperglycemia causes irregularities in platelet function, leading to prolonged bleeding.

Additionally, the presence of persistent hyperglycemia significantly impacts wound healing by increasing inflammation through the alteration of neutrophil and macrophage activity, thereby delaying the healing process. The activation of fibroblasts and keratinocytes is also impaired in diabetes, resulting in poor matrix deposition, reduced angiogenesis, and prolonged wound healing during the proliferative phase. Furthermore, with the impairment in collagen accumulation, the tensile strength of wounds is diminished, which then hinders tissue regeneration. Therefore, persistent hyperglycemia affects all stages of wound healing, contributing to delayed healing and further exacerbating the risk of microbial contamination.

2.3.5 Diabetic retinopathy

Every organ in the body plays an important role, and the eye is one of them, responsible for vision. The cornea and lens are essential parts of the eye, involved in capturing light and allowing it to focus on the retina, another crucial part of the eye. The retina works by converting light into electrical signals, which are then transported to the brain via the optic nerve. Structurally, the retina contains two important photoreceptors, rods and cones, which play a crucial role in detecting light and color. Additionally, the retina is responsible for transporting essential nutrients and oxygen to its cells through the mesh network of blood vessels present in it (Chong et al., 2024). Under normal or stable glucose conditions, the retinal blood vessels ensure the efficient functioning of endothelial cells, maintaining the integrity of the blood-retina barrier and preventing the leakage of fluids and proteins into the retinal tissues. Similarly, when glucose levels are stable, the formation of AGEs is significantly reduced, which diminishes oxidative stress and minimizes the risk of retinal blood vessel damage. Stable glucose levels not only prevent retinal damage but also regulate VEGF, ensuring controlled blood vessel formation for the efficient transport of oxygen and nutrients to the retina (Zhang et al., 2024). Under non-diabetic conditions, the retina remains healthy, with no structural or functional changes, thereby greatly reducing the risk of developing diabetic retinopathy. However, when exposed to prolonged high blood glucose levels, these conditions are reversed. Elevated glucose levels lead to increased oxidative stress and the formation of AGEs, which contribute to endothelial dysfunction. This dysfunction disrupts the blood-retina barrier, causing fluids and proteins to leak into the retinal tissues, resulting in retinal edema. As the condition worsens, newly formed blood vessels are fragile and inefficient in delivering oxygen to the retina, leading to hypoxic conditions. These hypoxic conditions further accelerate disease progression, potentially leading to irreversible vision impairment (Haydinger et al., 2023).

2.4 Current treatment strategies for managing diabetes and associated drawbacks

Individuals with diabetes are advised to adopt a nutritious diet and engage in regular physical activity to manage elevated blood sugar levels. However, in cases where diet and lifestyle adjustments prove ineffective in normalizing glucose levels, diabetic individuals are compelled to initiate anti-diabetic therapy. Numerous anti-diabetic medications have been used in clinical practices to manage hyperglycemia; however, their prolonged use can lead to several side-effects.

2.4.1 Metformin

Metformin (or 3-(diaminomethylidene)-1,1-dimethylguanidine) is a hypoglycemic drug that falls under the class of Biguanide (BCS class III drug). This chemical compound exhibits its anti-diabetic potential by inhibiting gluconeogenesis in the liver, thereby causing a decline in the level of blood glucose by improving insulin sensitivity (Madiraju et al., 2018). For instance, a recent report has shown that metformin at the dose of 1000 mg/kg causes a significant decline in glucose levels and substantially improves hepatic and renal functioning in alloxan-induced diabetes in albino rats (Almuttairi, 2023). Another research showed the treatment of metformin to male C57BL/6J diabetic mice at varying dosages i.e., 60 mg/kg, 200 mg/kg and 400 mg/kg showed a dose-dependent reduction in blood glucose levels by inhibiting intestinal glucose transport (Horakova et al., 2019). Besides anti-diabetic activity, metformin has been reported to exert a vast array of therapeutic applications viz, anti-cancer, anti-inflammatory, and antioxidant (Fan et al., 2021; Cheng et al., 2021; Fan et al., 2020). Since this anti-diabetic molecule holds the potential to lower glucose level by improving insulin sensitivity; the clinical efficacy of this drug is limited to its low permeability, short half-life (approximately 2-6 h), poor absorption, low bioavailability (40–60%), and short elimination half-life (1.5-1.6 h) (Mady et al., 2021; Kinaan et al., 2015; Song, 2016; Bouriche et al., 2021). To maintain an effective plasma concentration, this clinically approved drug is advised to give repeatedly at high doses (2.5 g/day), which further causes serious gastrointestinal side effects (Chinnaiyan et al., 2019). This highlighted that there is a need to look for an alternative strategy that helps to improve its clinical therapeutic efficacy by overcoming drawbacks associated with it.

2.4.2 Acarbose

Acarbose is a microbial-derived compound produced through the fermentation of *Actinoplanes* species. This chemical compound works as an α -glucosidase inhibitor by

delaying the digestion and absorption of carbohydrates, thus preventing the breakdown of complex carbohydrates into simple sugars, thereby reducing postprandial glucose spikes. Acarbose acts locally in the gastrointestinal tract with minimal systemic absorption, acarbose lowers the risk of systemic side effects. However, undigested carbohydrates fermented by gut microbiota in the colon can cause gastrointestinal issues like bloating, flatulence, and diarrhoea (DiNicolantonio et al., 2015).

Acarbose plays a significant role in managing type 2 diabetes, especially as an adjunct therapy. For instance, a previous study showed that this chemical compound at 40 mg dose can effectively lower the blood glucose levels in STZ-infused diabetic rats (Wright et al., 1998). Another research showed that C57BL/6 mice-infused with STZ treatment (40 mg/kg for 5 consecutive days) showed significant reduction in blood glucose levels, improved insulinitis, reduction in inflammatory markers and cytokine-induced apoptosis, upon receiving acarbose therapy at a dose of 50 mg/kg dose for 2 consecutive weeks (Zhao et al., 2020). Despite its potential in lowering blood glucose levels, the use of this anti-diabetic medication calls it in question. For example, the short half-life of this drug is very less (only 2 hours) by which the drug is not able to reach its target site, and as a result, the drug requires frequent administration around 3-4 times per day with a dose of 25-100 mg. This repetitive administration of acarbose cause several side-effects, including gastric upset, abdominal distension, and diarrhoea. For instance, in controlled clinical trials, more than 50% of the patients, who were on Acarbose therapy, report these side effects (Rosak & Mertes, 2012). This highlighted that there is a need to look for an alternative strategy that helps to improve its clinical therapeutic efficacy by overcoming drawback associated with it.

2.4.3 Glibenclamide

Glibenclamide (or 5-chloro-N-[2-[4-(cyclohexylcarbamoylsulfamoyl) phenyl]ethyl]-2-methoxybenzamide) is a clinically used oral anti-diabetic drug belonging to the class of sulphonylureas (BCS class II). This chemical compound works as an anti-cancer agent by inhibiting the proliferation of MDA-MB-231 breast cancer cells followed by induction of cell cycle arrest at the G0/G1 phase (Vaculikova et al., 2019). In addition, this drug behaves as an anti-diabetic by blocking ATP-sensitive potassium channels and depolarization of pancreatic beta cells (Luzi et al., 1997). Besides these medicinal applications, poor solubility (4 mg/L), low oral bioavailability (40-45 %), and short half-life (approximately 3-4 h) of Glibenclamide affect its clinical applications (Philip et al., 2009; Budiman et al., 2019; Goudanavar et al., 2010). The drawbacks associated with glibenclamide necessitate the seek

to look for an effective alternative, one of which is the development of glibenclamide-based nanoformulations with improved bioavailability.

2.4.4 Glimepiride

Another oral anti-diabetic medication that belongs to the third generation of sulfonylureas called glimepiride is clinically used to treat type 2 diabetes. This drug acts as an anti-diabetic agent by directing pancreatic β -cells to increase insulin secretion. In essence, it achieves this by blocking ATP-sensitive potassium channels in pancreatic β -cells, resulting in cell depolarization and, consequently, a reduction in blood glucose levels (Lodato et al., 2023). Extensive preclinical evaluations have been conducted using animal models of diabetes to establish its protective role in mitigating diabetes and diabetes-induced oxidative stress. For instance, Krauss and colleagues conducted a study indicating that administering glimepiride orally to STZ-induced diabetic rats for seven consecutive weeks at a dosage of 200 mg/kg resulted in a substantial reduction in glucose levels up to 70.1 %. Furthermore, this treatment strategy resulted in enhanced antioxidant parameters and a decrease in malondialdehyde (MDA) levels (Krauss et al., 2003). Zhou et al. conducted a study demonstrating that treatment with glimepiride for a continuous period of 2 weeks, at a dosage of 0.5 mg/kg, resulted in reduced glucose levels and improved insulin levels in animals subjected to an HFD-fed, STZ-induced diabetic model. Furthermore, the administration of glimepiride to diabetic rats led to an upregulation of GLUT4 expression in both muscle and adipose tissues, suggesting a possible link to insulin receptor-independent activation of IRS1/Akt (Zhou et al., 2019). Despite the therapeutic potential of this drug in controlling glucose levels, its clinical effectiveness is limited by its elimination half-life of only 5 h and poor water solubility (<0.004 mg/ml or 1.6 μ g/ml), resulting in a poor dissolution profile (Li et al., 2016). This emphasizes the importance of investigating alternatives to boost the clinical therapeutic effectiveness of glimepiride through enhancements in its water solubility.

2.4.5 Sitagliptin

Sitagliptin is a hypoglycemic agent belonging to the dipeptidyl peptidase-4 inhibitor class. It enhances the action of incretin hormones, such as glucagon-like peptide-1 and gastric inhibitory polypeptide, by preventing their degradation. These hormones promote insulin secretion from pancreatic β -cells and suppress hepatic glucagon release, thereby lowering blood glucose levels (Godinho et al., 2015). A study by Nagao et al. (2023) involving 176 elderly patients (aged 65-80 years) with moderately controlled type 2 diabetes demonstrated

that sitagliptin significantly reduced fasting plasma glucose, HbA1c, and glycated albumin levels compared to a control group, without serious adverse effects. Similarly, a clinical trial (ClinicalTrials.gov: NCT01177384) confirmed that sitagliptin, when combined with acarbose, was well-tolerated and significantly improved glycemic control over 24 weeks in patients with insufficient response to acarbose monotherapy (Wang et al., 2017).

Despite its therapeutic efficacy, sitagliptin is associated with several side effects. Common adverse events include headaches, upper respiratory tract infections, and nasopharyngitis (Zaghloul et al., 2018). Gastrointestinal symptoms such as nausea and diarrhoea have also been reported (ClinicalTrials.gov: NCT01703637; Zhou et al., 2019). Moreover, sitagliptin has been linked to an increased risk of acute pancreatitis. A retrospective cohort study using Taiwan's National Health Insurance database reported a higher incidence of acute pancreatitis in sitagliptin users (224.0 vs. 168.4 per 100,000 person-years) with an adjusted hazard ratio of 1.59, peaking in the initial months of therapy (hazard ratio 8.10) and declining thereafter (Tseng, 2015). Although associated with side effects, sitagliptin remains a clinically important option for managing type 2 diabetes. Further advancements in drug delivery and formulation may help reduce adverse effects and enhance therapeutic outcomes.

2.5 Challenges in current treatments for diabetes

A large number of challenges persist in managing the diabetes effectively, despite the availability of treatments. For example, the commonly prescribed hypoglycemic drugs for diabetes, such as metformin, acarbose, glibenclamide, glimepiride, and sitagliptin, often possess limitations that hamper their long-term clinical applications. For instance, metformin, while effective at improving insulin sensitivity and lowering glucose levels, experiences low bioavailability (40-60%), by which the drug requires repetitive administration to maintain therapeutic levels. Frequent dosing not only causes poor patient compliance but also leads to the onset of several gastrointestinal side effects, such as nausea and diarrhoea (Drzewoski & Hanefeld, 2021).

Similarly, acarbose, an α -glucosidase inhibitor, can cause gastrointestinal discomfort, such as bloating and flatulence, due to fermentation by gut microbiota. Additionally, its short half-life often necessitates repetitive administration of large doses, which further contributes to patient non-compliance (Barber et al., 2021). Glibenclamide, another widely used anti-diabetic drug, suffers from poor solubility and low oral bioavailability, limiting its absorption and clinical efficacy. Glimepiride, although a promising drug in controlling glucose levels, also faces similar challenges, such as poor water solubility and a short elimination half-life.

Sitagliptin, while effective in improving glycemic control, is associated with side effects, including headache, respiratory infections, and a potential risk of acute pancreatitis. These challenges underline the pressing need for more effective strategies to enhance the therapeutic efficacy of these drugs, reduce side effects, and improve patient compliance.

2.6 Introduction to drug delivery and routes of drug administration

The route of drug administration significantly influences drug toxicity, efficacy, and biological outcomes. Each delivery method has specific advantages and limitations that guide formulation design. Oral administration remains the most favorable route due to its convenience, patient compliance, prolonged shelf life, sustained release potential, and ability to enhance immune responses (Lin et al., 2017). In contrast, parenteral routes like intramuscular and intravenous injections bypass the gastrointestinal tract but are associated with discomfort, needle phobia, infection risks, muscle damage, and the need for trained personnel (Acharya et al., 2014; Baryakova et al., 2023). While alternative routes such as transdermal and topical delivery offer rapid or localized effects, they are less suitable for chronic conditions like diabetes. Oral delivery of drugs and nanoformulations remains optimal for long-term diabetes management due to its balance of efficacy, safety, and patient acceptability (Pardhi et al., 2024).

2.7 Barriers associated with drug delivery

Oral drug delivery is widely favoured due to patient compliance, non-invasiveness, cost-effectiveness, and ease of large-scale production in various forms (capsules, tablets, powders) (Bhutani et al., 2021). It treats both systemic and local gastrointestinal conditions but faces significant physiological and biochemical barriers, including acidic gastric pH, intestinal microbes, and mucus, which reduce drug half-life, bioavailability, and solubility (Figure 2.4).

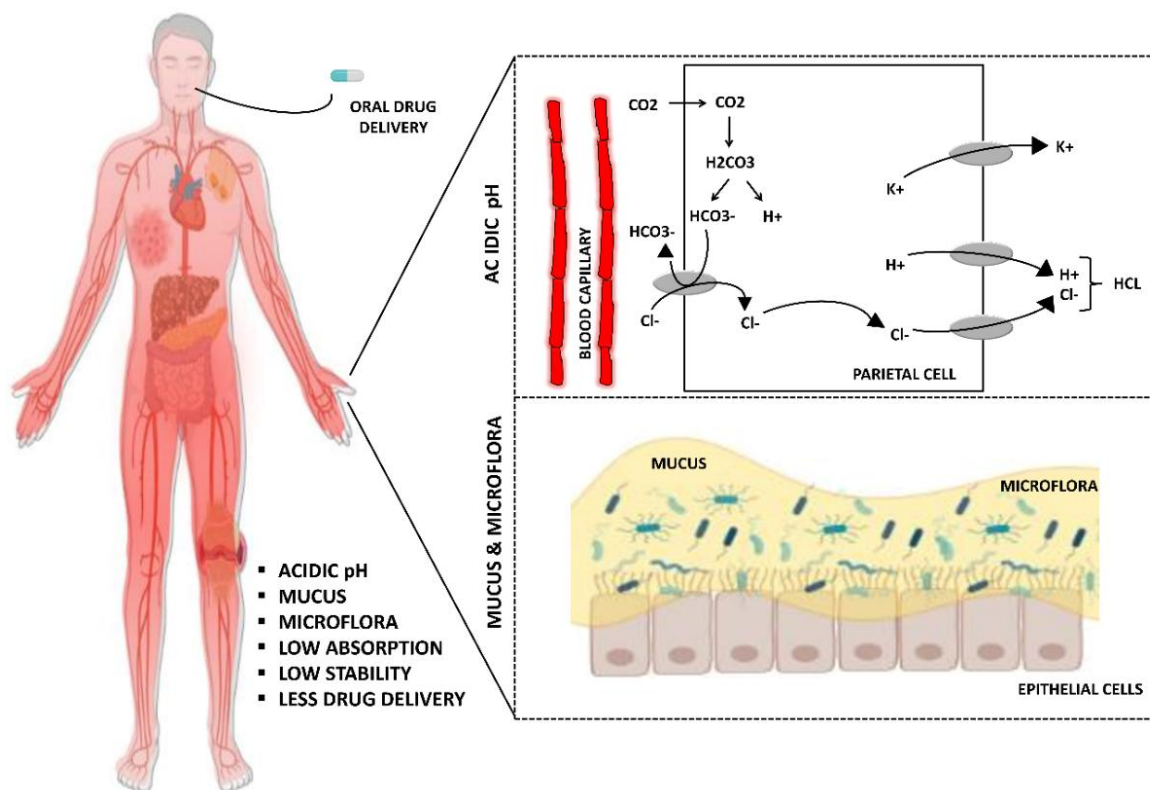


Figure 2.4: Schematic representation of biological barriers in oral drug delivery including gastric pH and intestinal microflora.

2.7.1 Anatomical factors

The gastrointestinal tract comprises the oral cavity, esophagus, stomach, small intestine, and colon, each influencing drug absorption differently. The oral mucosa offers good permeability but limited surface area and enzymatic degradation challenges absorption (Garg & Gupta, 2023). The esophagus has minimal impact due to short drug residence time. The stomach's acidic pH (1.5-2) and enzymes like pepsin facilitate digestion but degrade many drugs, lowering their efficacy (Sensoy, 2021; Stillhart et al., 2020). The small intestine, with extensive villi and microvilli, is the primary site for absorption but faces enzymatic and mucosal barriers that limit bioavailability (Ding et al., 2024). The colon has a higher pH and longer residence time with lower enzymatic activity, but drug metabolism by gut microflora varies between individuals, complicating colon-targeted delivery (Crouwel et al., 2020).

2.7.2 Biochemical factors

The gastrointestinal tract pH varies from acidic in the stomach to alkaline in the colon (pH 1–8), influencing drug stability and absorption (Wei et al., 2024). Enzymatic degradation, especially of protein drugs, limits bioavailability. The colon harbors over 400 microbial

species producing enzymes that metabolize xenobiotics and polysaccharides uniquely, which can be exploited for targeted delivery (Hu et al., 2023).

2.7.3 Physiology factors

The epithelial cell layer and mucus barrier limit drug permeation. The phospholipid bilayer favours lipophilic absorption but restricts hydrophilic drugs due to tight junctions (Azman et al., 2022). Mucus, a viscous gel of mucins and glycoproteins linked by disulfide bonds, acts as a physical and enzymatic barrier to foreign substances, protecting the epithelium but hindering drug absorption (Bedi et al., 2021; Sheng & Hasnain, 2022). This indicates that encapsulating drugs in polymers can enhance their passage through these barriers, thereby improving systemic delivery and therapeutic efficacy.

2.8 Need for improved solubility and pharmacokinetics of clinically affected anti-diabetic medications

Diabetes remains a significant global health challenge requiring effective anti-diabetic therapies. However, approximately 75% of novel drug candidates and 40% of marketed drugs exhibit poor water solubility, resulting in slow dissolution but rapid release in the gastrointestinal tract, leading to suboptimal drug delivery (Di et al., 2012). Many clinically used anti-diabetic drugs suffer from poor solubility and low oral bioavailability, necessitating high and frequent dosing that often causes gastrointestinal side effects. Nano-delivery systems, particularly those utilizing natural polymers, offer promising solutions to enhance drug stability, solubility, and pharmacokinetics while minimizing adverse effects (Pardhi et al., 2024). Although nanotechnology holds great potential for improving anti-diabetic drug efficacy and patient compliance, challenges such as scalability and safety concerns limit its clinical translation. Plant-based polysaccharides have gained attention for their ability to protect drugs and prolong stability, yet full clinical optimization remains elusive. Therefore, continued advancement in nanodelivery platforms is essential to overcome these barriers and effectively reduce the global diabetic burden.

2.9 Guar gum in drug delivery: Advantages over other polymers

Guar gum is heavily used by pharmaceutical industries to make an effective nanoformulation with potential to beat the obstacles faced by drug when taken orally. Any polymer used for drug delivery must be a good binder (highly adhesive), stabilizer, disintegrating agent, and emulsifier (Mudgil et al., 2014). Guar gum meets all the conditions to act as an efficient

polymer and to overcome the barriers of drug delivery. Studies have proven that consumption of guar gum helps in controlling diabetes. Additionally, guar gum also possesses activities like anti-inflammatory, anti-proliferative and anti-microbial activities (Hamdani et al., 2018). Among all other reagents, guar gum shows high solubility in water and its endosperm contains galactomannan gum, which combines with water to form a viscous solution. In the food industry, this viscous solution creates a lot of constraints when consumed as dietary fiber (Thombare et al., 2016). In pharmaceutical industries its perpetual use has been to modify the excipient profile with the help of grafting, network formation, and derivatization which allow its use in drug delivery (Prabaharan, 2011).

Reports have revealed that when a mixture of guar gum and water is heated, the water molecule that surrounds the guar gum loses its order (Hasan & Abdel-Raouf, 2018). Due to which the conformational changes occur, and the viscosity of guar gum decreases in hot water. When blended with water, the viscosity rate of guar gum increases, reducing the employment and effectiveness of lipids and proteins. These aspects fundamentally contribute to disturbances in digestion and absorption of nutrients, commencing gastric problems. And, thus it is advisable to use the partially hydrolyzed guar gum because of its low solubility to avoid this (Yoon et al., 2008). Partially hydrolyzed guar gum appears as a fine white powder in its dried form. The low viscosity does not interfere with the digestion and absorption of nutrients so, gastric problems do not occur. Treatment of guar gum with β -endo mannanase enzyme results in the formation of partially hydrolyzed guar gum (Russo et al., 2015). Due to its low viscosity, partially hydrolyzed guar gum is also highly used in the food industry as a dietary fiber. Drug delivery can be controlled and improved by removing the key constraints such as prolonging the drug release time and preventing degradation before reaching the target site. To achieve controlled drug release, guar gum can be used as a tablet binder and thickening agent (Chavda et al., 2012). Guar gum has been used extensively all over the world and its nanoformulations are considered as excellent carrier for controlled and sustained drug delivery (Patra et al., 2018).

While natural polymers such as chitosan, alginate, and pectin are widely used in drug delivery, guar gum offers several distinct advantages that make it a superior choice for oral and colon-targeted formulations. Chitosan is primarily soluble in acidic media and exhibits limited stability in neutral or basic conditions, which may restrict its application in the small intestine and colon (Aranaz et al., 2021). Alginate, although biocompatible, requires divalent cations for gel formation and exhibits rapid swelling in aqueous environments, which can lead to premature drug release (Zhang et al., 2024; Khatibi et al., 2024). Pectin, on the other

hand, often needs chemical modification or high concentrations to achieve sufficient gel strength for controlled release (Huang et al., 2025; Jung et al., 2013). In contrast, guar gum demonstrates high water solubility across a broad pH range, forms highly viscous solutions at low concentrations, and is resistant to enzymatic degradation in the stomach and small intestine, allowing it to reach the colon efficiently (Garg & Gupta, 2023). Its ability to form hydrogels, matrix tablets, and nanoparticles ensures controlled drug release, enhanced bioavailability, and stability of drugs under gastrointestinal conditions (Vegad et al., 2023). These properties position guar gum as a particularly effective polymer for applications in colon-targeted delivery, sustained release formulations, and biomedical therapies where drug protection and site-specific delivery are critical.

Colon-targeted drug delivery witnesses to be very valuable for the treatment of colon-related diseases such as inflammatory bowel disease and colorectal cancer (Philip & Philip, 2010). These diseases can only be overwhelmed by targeting the colon directly as there is no systemic adverse effects. Small intestinal delivery of nanoparticles at low doses corrects the local and systemic absorption which are moderately beneficial (Date et al., 2016). The poor solubility, low drug resistance at acidic pH as well as low bioavailability can be improved by preparing the polymer-based nanoformulations. The combinational approach of using guar gum nanoformulation with other drugs has shown excellent results by increasing the bioavailability, stability, and delay in degradation. Therefore, the present review attempts to shed light on the recent advances in the development of guar gum based nanoformulations to improve the bioavailability and stability of the poorly soluble drugs. Data obtained from *in vitro* and *in vivo* studies suggest that guar gum is an excellent carrier for achieving controlled drug release, particularly for targeting inflammatory, cancer, colon, and brain-related disorders.

2.10 Source and structure of guar gum

Guar or cluster beans are a rich source of guar gum. The botanical name of this plant is *Cyamopsis tetragonoloba* (Sharma et al., 2017), and the plant refers to the Leguminosae family. In the endosperm of guar beans, guar gum is present in the form of galactomannan. Additionally, India is the only country in the world that contributes 80% of guar gum production (Mudgil et al., 2014). The Indian economy benefits exceedingly from the cultivation of guar gum. Natural gums have no definite structure. In short, gums contain an indefinite number of sugar molecules, due to which they fall under the category of polysaccharides. Monosaccharides composition plays an important role in the guar gum

(Navarro et al., 2019). Monosaccharides are crystalline solids and are highly soluble in water. The high-water solubility of guar gum also depends on its monosaccharide composition. Mannose and galactose are two major monosaccharides present in guar gum, due to which they are also called galactomannan. Guar gum is composed of two D-mannose units that are linked by β -1 $\rightarrow$ 4, which formed the linear polymer backbone, to which D-galactopyranosyl units are attached by (Hongbo et al., 2012). The chemical structure of guar gum is depicted in Figure 2.5.

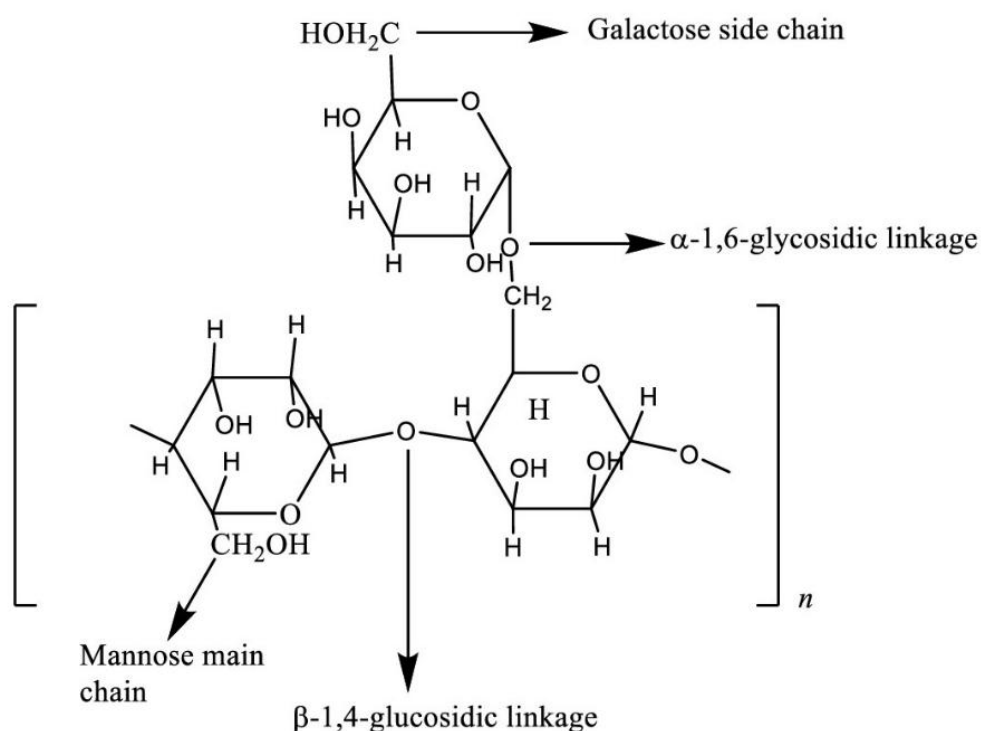


Figure 2.5: Structure of galactomannan (guar gum)

2.11 Physio-chemical properties of guar gum

The biological properties of guar gum are predominantly dependent on the properties of solubility medium. Guar gum relatively shows high solubility in cold water. Addition of guar gum in non-polar solvents results in the formation of weak hydrogen bonds but its addition to polar solvent results in the formation of strong hydrogen bonds. As the pH and particle size reduces, the viscosity and dissolution of guar gum increases. Other than pH and particle size, increasing temperature also contributes to the viscosity and dissolution of guar gum, whereas the presence of salts and sucrose tends to lessen the hydration rate of guar gum.

2.11.1 Rheology

The property that deals with the material flow and deformation upon applying an external force is called the rheology. Guar gum is a high molecular weight polysaccharide that shows pseudoplastic and shear-thinning characteristics upon mixing with the aqueous medium (Chenlo et al., 2010). A report by Chang et al. showed that presence of these characteristics reduced the viscosity of guar gum with the elevation in shear thinning rate (Chang et al., 2017). In general, as the concentration and molecular weight of polymer increased, the shear-thinning characteristic of guar gum in aqueous medium also increased (Zhang et al., 2020). A study by Shobha and colleagues reported that 1% concentration of guar gum in aqueous solution, the loss modulus (G'') was dominated by storage modulus (G') in higher frequency but under low frequency rate, the storage modulus (G'') was found to be dominated by loss modulus (G'') (Shobha & Tharanathan, 2009). Later, it was revealed that as the time passes, the storage modulus and loss modulus tend to reduce (Chenlo et al., 2010).

2.11.2 Viscosity

The guar gum when dissolved in cold water apparently forms the highly viscous solution due to its hydration property. Similar to other gums, various factors such as time, pH, temperature, and concentration are responsible for the viscosity of guar gum.

2.11.3 Hydration rate

A report by Wang and colleagues showed that hydration rate of guar gum depends on its particle size (Wang et al., 2006). In order to achieve the maximum viscosity, the hydration period of 2 h is required for guar gum solution.

2.11.4 Hydrogen bonding activity

Structurally, guar gum contains hydroxyl group that contributes to the formation of hydrogen bonds. Hydroxypropyl can effectively replace the hydroxyl groups in guar gum structure leading to the steric hindrance and instability of hydrogen bonds (Li et al., 2020).

2.11.5 pH

The non-ionic and uncharged feature stabilizes the guar gum solution at broad pH range from pH 1.0 to 10.5. Variation in pH leads to the reduction in hydration rate of guar gum (Wang et al., 2000); however, viscosity of guar gum is not influenced by the pH. A pH value less than 4 and higher than 10 slows down the hydration rate of guar gum; whereas hydration rate

of guar gum was found to be high between 8 and 9 pH ranges (Carlson et al., 1962).

2.11.6 Temperature

The rate of hydration and viscosity are greatly influenced by the temperature. It had been found that under high temperature, guar gum solution attains high viscosity than preparations at lower temperature (Casas et al., 2000). The ideal temperature range for dispersed solutions of guar gum is 25-40 °C, whereas 25 °C is noted as the suitable temperature for 0.5% (w/w) solution of guar gum to gain the maximum viscosity (Gyawali & Ibrahim, 2016).

2.12 Recent advances in biomedical applications of guar gum for disease amelioration

It is quite difficult to control the release of drugs in oral delivery as the drug faces constraints like acidic pH and intestinal enzymes etc. Due to the short stability period, the drug is not able to survive long and degrades before reaching its target site. Polymers play an important function in protecting the drug and helping it reach its target site. A plethora of studies of applications of nano-strategies are available in the literature in the context of designing guar gum based nanoformulations where poorly soluble drugs have been coated with guar gum either in the form of nanoparticles, nanofibers, nanotubes, nanorods, nanoemulsions or nanocapsules to improve the bioavailability and controlled drug delivery. Figure 2.6 demonstrates the mechanism of drug-loaded guar gum nanoformulations in reducing the drug degradation and achieving controlled release rates by improving drug stability and bioavailability.

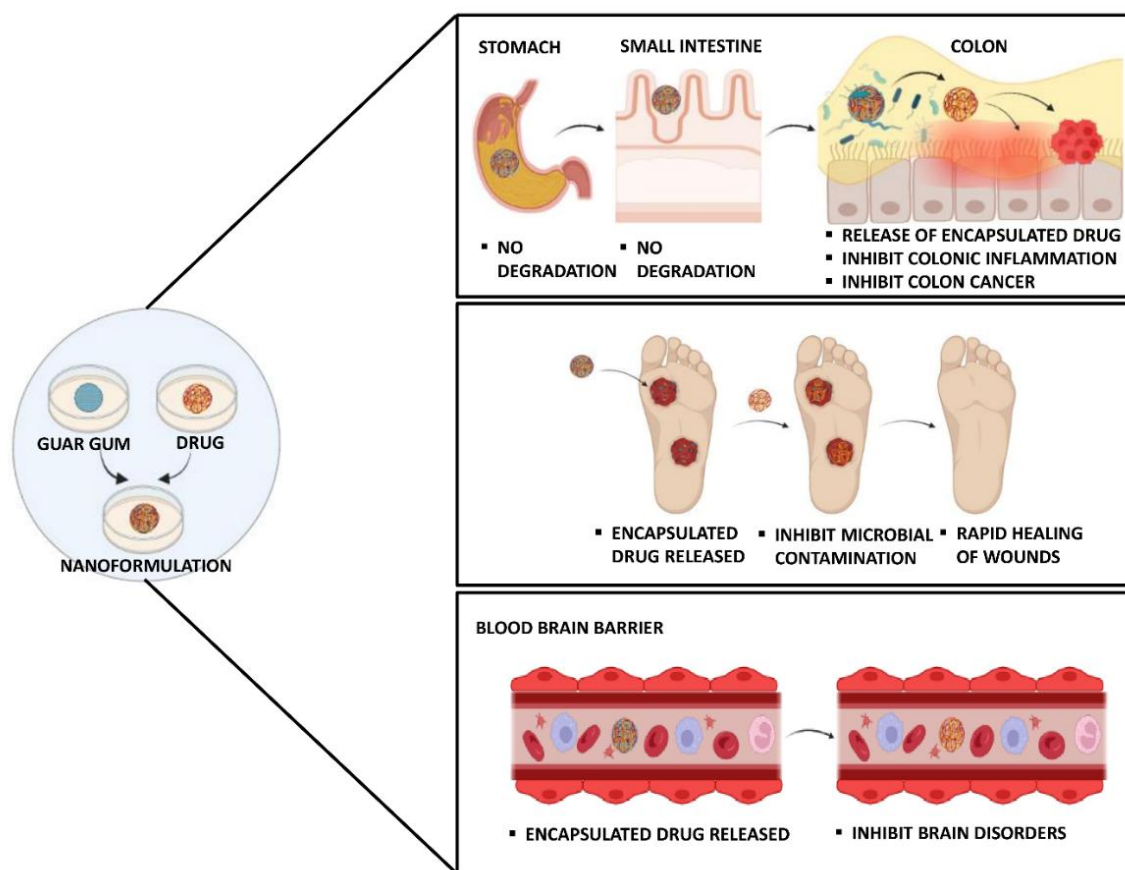


Figure 2.6: Schematic representation of drug-loaded guar gum nanoformulations achieving controlled release by slowing initial drug release before reaching the target site.

2.12.1 The role of guar gum based nanoformulation in gastrointestinal tract

Among hydrophilic polysaccharides, only guar gum has attained the ample attention due to its properties to prevent the drug degradation in acidic pH of stomach, resistance towards colonic microflora, stabilizing the drug and exhibit the controlled release of drug in its targeted site by enhancing the bioavailability. Several theories have been claimed that, non-ionic and uncharged features of guar gum increased its stability in acidic pH of the stomach (Jani et al., 2009). Guar gum is a prolific agent which gains the attention of researchers by facilitating the release of drug in its targeted site by crossing the prime barrier i.e., acidic pH and digestive enzymes. Thus, pH-responsive guar gum based nanoformulations are effective in improving the bioavailability and stability of drugs in the gastrointestinal tract. For instance, 5-aminosalicylic acid loaded guar gum-based matrix tablets were synthesized by Mahto and colleagues using the graft copolymerization reaction. These tablets are capable to restrict the release of 5-amino salicylic acid in acidic pH of stomach and helps in improving drug delivery (Mahto et al., 2019). On the other hand, using free radical cross-linking polymerization

reaction, Jalababu et al. prepared the hydrogels using NAcryloyl-L-phenylalanine (APA) and guar gum to produce the graft copolymer (GG-g-PAPA). Later, this graft copolymer was cross linked with 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), and 2-hydroxy ethyl acrylate (HEA) to form the novel pH- responsive GG-g-PAPA-cl-(PHEA-co-PAMPS) [GGAH] polymeric hydrogels. The swelling kinetics of this hydrogel was evaluated in distilled water which showed that at pH 7.4, this hydrogel shows its maximum swelling capacity whereas the swelling capacity decrease with the increased salt concentration (Jalababu et al., 2019).

It has been claimed that reduced swelling property of the hydrogels at low pH tends to slow down the release of drug, which are effective in surviving low pH and controlled drug delivery. In another study, scientists used the N, N-methylene-bis-acrylamide as a cross-linking agent to synthesize the novel pH-responsive guar gum/poly (acrylamide- coacrylamidoglycolic acid) hydrogel matrices. Later, these hydrogels were loaded with 5-fluorouracil, which is a potent anticancer drug. They examined the efficiency of this hydrogel on the basis of their drug release and swelling kinetics and showed that at low pH, the swelling kinetics was tremendously decreased which ultimately slower down the release of 5-fluorouracil from the hydrogel, hence pH-responsive hydrogels are suited for controlled drug delivery in cancer therapies (Reddy et al., 2018). Another series of a pH-responsive grafted guar gum with amino acid lysine- β -cyclodextrin loaded 5-fluorouracil hydrogels were synthesized by the Prabhakar and colleagues. Later these hydrogels were evaluated for their potential to releasing the drug in a controlled manner. They showed that among acidic and basic conditions, the anticancer drug, 5-fluorouracil is dramatically released in acidic conditions. Moreover, they also revealed that this hydrogel at acidic environment can potentially swells and display the delivery of drug in a controlled way (Prabhakar et al., 2018).

Seeli et al. focused on synthesizing the novel pH-responsive hydrogel of glycol dimethacrylate (EGDMA) that was cross-linked with oleate- graft poly (methacrylic acid) and encapsulated with ibuprofen. They found that guar gum oleate moieties simultaneously increased the efficiency of drug loading and thereby improves the drug delivery. At pH 7.4, these hydrogels were found to increase the degree of swelling and increase the retention period of drug as compared to pH 1.2 (Seeli & Prabhakaran, 2017). Sodium Wyoming (Swy-2) and Montmorillonite are two different clay minerals present in the nature. Scientists have used these clay minerals to form a guar gum based novel nanocomposite and load Ibuprofen in to these nanocomposites. Using an *in vitro* approach, these nano composites were evaluated for their drug release potential. Analysis of their study showed that these nanocomposites released the

ibuprofen in a controlled manner at pH 7.4, suggesting the potential of guar gum-based nanocomposites to achieve the controlled delivery of drugs (Dziadkowiec et al., 2017).

2.12.2 Role in colon-related disorders

The longest part of the large intestine which withdraws the water, nutrients, and electrolytes required for the partially digested food is called the colon. Patients who suffer from colonic disease have a high level of ROS and inflammatory cytokines. The increased level of ROS inhibits the action of antioxidants that directly contributes to the disease severity (Alzoghaibi, 2013). Damaged tissues and cells secrete the chemicals that generate the inflammation (Garg et al., 2020). Pathogens present in the intestine produce toxins that increase the permeability of epithelial cells and inflammation in the intestinal area. Elevation in inflammation and permeability alters the concentration of drugs in colonic mucosa and results in the development of inflammatory bowel disease such as Crohn's disease and ulcerative colitis (Hendrickson et al., 2002). Ulcerative colitis has a definite location, it occurs only in the large intestine but no such location is defined in Crohn's disease, for example, it could be somewhere in the digestive tract. In ulcerative colitis, the inflammation is confined to the mucosa but in Crohn's disease, the inflammation affects the entire bowel wall (Qin, 2013). When the intestinal wall becomes inflamed, fistulas form in the internal organs and skin. Inflammation in the mucosa causes many biochemical changes in immune cells. These changes include infiltration of neutrophils, dendritic cells, macrophages, and lymphocytes (Garg et al., 2020). Biochemical changes in immune cells increase the permeability of epithelial layer present in the colon and lead to diarrhoea.

It is well known that inflammation in the intestinal lining frequently increases the danger of rupture of tissues around the anus, which can be considered a prime reason for the occurrence of anal fissure. Based on this condition, Brilliantino et al. studied the effect of partially hydrolyzed guar gum in chronic anal fissure patients. The patients were treated with the topical application of glyceryl trinitrate followed by administration of a 5 g dose of partially hydrolyzed guar gum and found that gum effectively reduced the fissures. Partially hydrolyzed guar gum reduces the inflammation in the lining of the intestines and hence acts as a prolific agent against inflammation, suggesting its role in preventing the inflammatory bowel disease (Brillantino et al., 2014). Naito et al. investigated the potency of partially hydrolyzed guar gum on colitis-induced mice and showed that partially hydrolyzed guar gum reduces the secretion of neutrophils. In addition, it also reduces the effects of inflammatory markers such as TNF- α

which helps in preventing mucosal inflammation. In addition to anti-inflammatory, guar gum also works well as an antioxidant agent. For example, it had been observed that amount of thiobarbituric acid reactive substances were increased in the experimental model of colitis due to the administration of dextran sulfate sodium, but after the treatment of guar gum, the quantity of these reactive substances decreased and hence it behaves as an antioxidant. The findings of this research depict that guar gum inhibits the inflammatory events and could be a promising candidate for improving drug delivery in inflammatory disorders (Naito et al., 2006).

Jhundoo and colleagues studied the effects of acacia and guar gum in an animal model of murine experimental colitis and showed that both gums are effective to reduce the expression of a carrier of inflammation i.e. TNF- α , IL-1 β , NF-kB, and IL-6. Treatment of these gums with 5-amino salicylic acid exhibits good results in the murine model while treatment of acacia significantly improved the inflamed tissues as compared to guar gum. The presence of negatively charged ions in the backbone of acacia sticks to the inflamed tissues and improves the inflammation in tissues via decreasing the level of inflammatory markers and hence proves potent anti-inflammatory than guar gum. Therefore, these results suggest that guar gum can be used as a polymer against the inflammatory bowel diseases as no degradation had been observed with the conjugation of guar gum (Jhundoo et al., 2021). Similarly, the role of partially hydrolyzed guar gum was also investigated by Takagi et al. using TNBS-induced colitis model. They found that colonic damage significantly reduced during treatment of colon with partially hydrolyzed guar gum. Meanwhile, partially hydrolyzed guar gum reduced the levels of TNF- α and myeloperoxidase that were significantly increased due to TNBS administration. Simultaneously, necrosis begins in the animals only after the administration of TNBS, but treatment with partially hydrolyzed guar gum reduces the necrosis and thus prevents the onset of inflammatory events in experimental models, suggesting that partially hydrolyzed guar gum can be used against inflammatory ailments (Takagi et al., 2016).

Similarly, another group of researchers formulated the cross-linked fluticasone tablets for controlled release in the colon. Later these tablets were cross-linked with chitosan-carboxymethyl-guar gum complexes. Administration of these tablets in TNBS induced ulcerative colitis in rats' results in significant improvement. After experimental analysis, it was found that cross-linking of fluticasone tablets with guar gum and chitosan showed a better result by controlling the drug release in the colon region. Thus, it is recommended to use the guar gum in combination therapy with other polymers to prevent the degradation of a drug before reaching its target site (Kumar et al., 2011). Sharma and colleagues worked on the

microparticles of embelin which were loaded with the guar gum, and investigated their pharmacological potential against ulcerative colitis through an *in vivo* study. Administration of these loaded micro particles inhibits the pathogenesis of ulcerative colitis. In addition, the shelf life of drug-loaded guar gum also increases the release rate of the drug at the target site. Simultaneously, the released frequency of embelin loaded guar gum was found to be 88.5 ± 3.8 % in 24 h, indicating a controlled rate of these microparticles in the target site, suggesting the function of guar gum in controlled delivery of drug (Sharma et al., 2018).

Flurbiprofen is a most widely used non-steroidal anti-inflammatory drug to lenience the colonic inflammation. The patients who were on flurbiprofen therapy, reported to have the gastric ulceration, and bleeding like side effects. This suggests that there is need to develop some nano formulation that effectively reduce the side effects of flurbiprofen and achieves the colonic-targeted drug delivery. Focusing on this aspect, flurbiprofen coated guar gum tablets were synthesized by Vemula and group. They showed that this formulation improves the release rate of flurbiprofen and inhibits its early degradation, confirming the potential of guar gum in controlling the drug release in colon (Vemula & Bontha, 2013). Albendazole is a broad spectrum anthelmintic drug which inhibits the formation of microtubules as well as prevents the infection caused by intestinal worms. Saraswathi et al. formulated an albendazole tablet containing guar gum and showed that as the concentration of guar gum increases, the amount of drug release in the colon drops. Additionally, the inclusion of guar gum over albendazole reduces its release into the gastrointestinal tract, although anaerobic conditions increase the release of drug which represents guar gum as an excellent polymer in the field of drug delivery (Saraswathi et al., 2010). Tenoxicam is widely used to suppress the inflammation caused by osteoarthritis and rheumatoid arthritis. But the drug is not stable in the gastrointestinal tract and hence it does not exhibit it therapeutic activity. This suggests that there is a need to coat this drug with a biocompatible polymer which prolongs the existence of drug in gastro intestinal tract by inhibiting the degradation. Therefore, leading group of researchers utilized the hydroxypropylmethylcellulose and guar gum to formulate stable tenoxicam tablets. They found that guar gum pre vents the release of the tenoxicam in the acidic environment of the stomach and allows a small amount of drug to be released in the small intestines and stomach. This suggests that the use of guar gum and hydroxypropylmethylcellulose helps the drugs to be release in a controlled way (Vagare, 2015).

2.12.3 The role of guar gum-based nanoformulations in skin regeneration

Preparation of a biocompatible wound dressing that releases drugs in a controlled manner and possesses antimicrobial activity always remains a huge challenge for researchers. Guar gum has been extensively used in combination with antimicrobial agents to inhibit the pathogenesis of microbial contamination in wounds. For instance, a novel hydrogel wound dressing containing guar gum and arabinoxylan was prepared, which was found to release the drug in a controlled manner (92%) and thus helps in faster healing of wounds by altering the activity of Gram-negative and positive bacterial strains (Khan et al., 2020). Ghosh and colleagues attempted to improve the release rate of ciprofloxacin by synthesizing ciprofloxacin-loaded carboxymethyl guar gum and gelatin hydrogels. They showed that these hydrogels are safe and help in slowing the release rate of ciprofloxacin, suggesting that they can be used for the treatment of wounds and antibiotic delivery (Ghosh et al., 2018). Likewise, Rao et al. prepared a self-healed series of hydrogels containing guar gum and alginate using varying concentrations of glutaraldehyde as a cross-linking agent. They found that the combination of guar gum and alginate as a hydrogel achieved high tensile strength and exhibited self-healing properties improved up to 95% (Rao et al., 2019). Another research showed that the development of novel L-alanine cross-linked grafted guar gum and acrylic acid hydrogel loaded with levofloxacin improved the release profile of levofloxacin up to 98%, hence demonstrating its role in achieving controlled drug delivery. At 90°C, these hydrogels were reported to be thermally stable, thus establishing their thermal resistance properties in self-healing drug delivery applications (Sharma et al., 2019).

Palem et al. grafted the guar gum with polyacrylamideglycolic acid and formed a network of dual-sensitive nanocomposite hydrogel systems. Later, these nanocomposite hydrogels were tested for the sustained release of 5-fluorouracil via silver nanocomposites in phosphate buffer and cross-linked grafted polymers. They showed that these nanocomposite hydrogels are effective against bacteria residing in the intestine and also exhibit controlled drug delivery by releasing 5-fluorouracil in a sustained manner. Moreover, these nanocomposite hydrogels are sensitive to pH and temperature and also display sol-gel behavior, suggesting the potential of such nanocomposite hydrogels in controlled drug delivery (Palem et al., 2020). Research showed the potential of guar gum-stabilized copper nanoparticle hydrogel in exhibiting self-healing and photothermal properties. Upon laser irradiation, these nanoparticles showed efficient self-healing with a sharp temperature rise. Moreover, these nanoparticles displayed

high antibacterial activity and low cytotoxicity, suggesting their role in photothermal therapy (Chen et al., 2017).

Likewise, the synthesis and antimicrobial potential of hydrogels from blends of chitosan/guar gum/poly(vinyl alcohol) were reported by Iqbal and colleagues. They showed that both guar gum and chitosan blended with polyvinyl alcohol release the drug in a controlled manner. Further, these hydrogels also exhibited antimicrobial activity against various bacterial agents such as *Pasteurella multocida*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* (Iqbal et al., 2020). Another group of pioneering scientists used self-assembling peptides and guar gum to obtain a biomimetic β -sheet-rich self-assembling peptide hydrogel. They reported that the formulated hydrogel was efficient in healing open wounds (Pugliese & Gelain, 2020). Similarly, Talodthaisong and colleagues contributed to improving the self-healing properties of hydrogel by synthesizing guar gum-coated silver nanoparticles. Later, they cross-linked this hydrogel with borax and loaded it with curcumin, and showed the inhibition of Gram-negative and positive bacteria upon treatment with this hydrogel. This concludes that guar gum-based hydrogel systems displayed antimicrobial properties due to high swelling and self-healing applications (Talodthaisong et al., 2020).

In 2020, scientists tried to prepare hydrophobically modified guar gum film. Later, they incorporated polyvinyl alcohol into the hydrophobically modified guar gum followed by the addition of two drugs, amoxicillin and gentamicin sulfate. This method yielded drug-loaded hydrophobically modified guar gum film, and the potential of this film was evaluated on wounded Wistar albino rats. Results of their investigation revealed that the modified guar gum film showed promising effects against the growth of bacterial (*S. aureus* and *E. coli*) and fungal (*Candida albicans*) strains. Moreover, these films resulted in complete recovery of the open wounds of rats within 15 days, demonstrating their importance in preventing microbial contamination and promoting faster healing of wounded tissues (Bajpai & Rai, 2021).

2.12.4 The role of guar gum-based nanoformulations in cancer

Curcumin is proven to be a prolific agent for cancer, particularly colorectal cancer but its poor solubility and breakdown in gastrointestinal tract reduced its medicinal application. This suggests that there is a need to formulate the curcumin loaded medicine protected by a biocompatible polymer such as guar gum to increase its bioavailability. Elias and colleagues formulate the curcumin matrix tablet coated with guar gum and attempted to test its therapeutic activity in colon cancer. They found that matrix tablet containing 40% guar gum helps the

curcumin to achieve the release rate of 91.1 % as compared to tablets containing 50 % guar gum. This research concludes that guar gum extends the release rate of curcumin and shows good results in limited amounts in colon cancer (Elias et al., 2010). In another research, scientists have developed the liquid solid tablets of curcumin coated them with guar gum and eudragit and showed that the dissolution and release of curcumin in small intestine and stomach were improved, suggesting the role of guar gum as a carrier to improve the drug release in cancer (Kumar et al., 2018).

Several theories have claimed that proteins lose their biological properties in acidic pH and are also degraded by intestinal enzymes. Moreover, the frequency of peptides that reaches to the systemic circulation is also decreased in oral delivery. So, to improve the delivery of proteins, protein delivery through buccal cavity is highly preferred. With this objective, Castro and group developed the novel nano formulation containing guar gum matrix loaded with poly(lactic-co-glycolic acid) and antihypertensive peptide against human buccal carcinoma. The treatment of novel peptide system on TR146 (human buccal carcinoma cells) showed that the release of antihypertensive peptide was slowed down due to the presence of guar gum film. Furthermore, even after the duration of 12 h, no toxicity was exhibited by this peptide system, highlighting that such novel peptide delivery system can effectively increase the bioavailability and therapeutic application of chemical compounds loaded in them (Castro et al., 2018). Later they incorporated alpha-casozepine peptide in PLGA-loaded guar gum nanoparticle film and evaluated their cytotoxicity parameters using TR146 buccal carcinoma cells. Their results revealed no sign of toxicity in cells treated with this peptide nanosystem. Moreover, the stability and permeability of alpha-casozepine was found to be increased in the gastrointestinal tract, which proves that guar gum matrix films are a good carrier to deliver the peptides (Castro et al., 2019). Another study demonstrated the synthesis of guar gum loaded gemcitabine nanoparticles with an approach to achieve the targeted delivery of gemcitabine in cancer therapy (Muthaiah & Ramachandran, 2019). The mesoporous silica nanoparticles loaded 5-fluorouracil and capped them with guar gum was also found effective in colon targeting drug delivery. *In vitro* colonic analysis demonstrated that capping of mesoporous silica nanoparticles with guar gum exhibit resistance towards the colonic enzymes and prevents its degradation in the gastrointestinal tract, suggesting the role of guar gum as a carrier to ameliorate the drug delivery therapies (Kumar et al., 2017). Scientists have also synthesized guar gum microparticles using doxorubicin and metformin hydrochloride. They showed that loading of guar gum improves the release rate of drugs by restricting their degradation. Moreover, they

also showed that guar gum-loaded microparticles are safe and show no toxicity (Kang et al., 2020).

Buccal cavity becomes an attractive mode to deliver the molecules whose size ranges from 500 to 1000 Da. Therefore, focusing on the delivery of bioactive molecules through buccal cavity Castro et al. prepared the film of guar gum with alginate bead loaded caffeine. Results showed that guar gum film enhance the caffeine bioavailability and shows no toxicity on human buccal carcinoma cells (TR146 cell lines), suggesting the role of guar gum film in enhancing the bioavailability of drugs (Castro et al., 2018). On the contrary, Dalei and colleagues used the tetraethyl orthosilicate and synthesized the fabricated carboxymethyl guar gum and polyvinyl alcohol. Later chrysin was loaded onto the hydrogels to confirm its potential in cancer therapy. They showed that at neutral pH, these hydrogels display their maximum swelling potential which helps the drug to release slowly. Moreover, the release of chrysin from hydrogel inhibits the breast cancer cell line (MCF-7 cells), thus confirming its capacity as suitable carrier in anti-cancer therapies (Dalei et al., 2021). Giri and colleagues prepared the nanocomposites and targeted against colorectal cancer. In their study, the 5-fluorouracil loaded nanocomposite was capped on outer side by guar gum where guar gum enhances the stability of 5-fluorouracil and restricts its degradation by colonic microflora, hence confirming the potential of guar gum in achieving the controlled drug release (Kumar et al., 2019). Pine trees and rosin glycerol esters are the two major sources of natural resin, called rosin. Therefore, an effort was made by Karakus et al. to synthesize the guar gum-Polyethylene glycol/Rosin glycerol ester nanoparticles. They showed that mixture of guar gum and polyethylene glycol is effective in improving the stability period of rosin ester nanoparticles via synergistic interactions. But the evaluation of this novel nanoformulation has not been tested yet which suggested that it need further evaluation in cancer models to check its efficacy in improving the drug delivery (Karakus et al., 2019).

2.12.5 The role of guar gum-based nanoformulations in brain-disorders

Studies have proved that borneol is effective in crossing the blood brain barrier but the low bioavailability of borneol limits its therapeutic application to combat the central nervous disorders (Zhang et al., 2017). Therefore, an attempt was made by Liu and colleagues to improve the bioavailability of borneol using guar gum as a natural polymer. Briefly, they developed a nanodelivery system by encapsulating borneol in vitamin E d- α -tocopheryl polyethylene glycol succinate-stabilized guar gum nanoparticles. The *in vitro* findings revealed

that the drug-loading capacity and dissolution rate of borneol were significantly improved by the guar gum-stabilized nanoformulation, which may serve as a potential strategy to enhance drug permeability across the blood-brain barrier (Liu et al., 2018). A team of researchers attempted to provide an idea on the stability of polymers by making a comparative study using guar gum, chitosan, and pullulan by considering this quest. Briefly, they synthesized the butylglyceryl-modified guar gum, chitosan and pullulan nanoparticles which were further loaded with doxorubicin, rhodamine B, and angiotensin II. The *in vitro* results of their experimental finding revealed that formulation containing butylglyceryl-modified guar gum showed the high loading capacity whereas the formulation containing pullulan showed the better release of drug with minimal toxicity inside the cells at high concentrations, suggesting the role of guar gum in high drug loading and pullulan in ameliorating the drug release to brain (Bostanudin et al., 2020). The biomedical applications of guar gum-based delivery systems are presented in Figure 2.7.

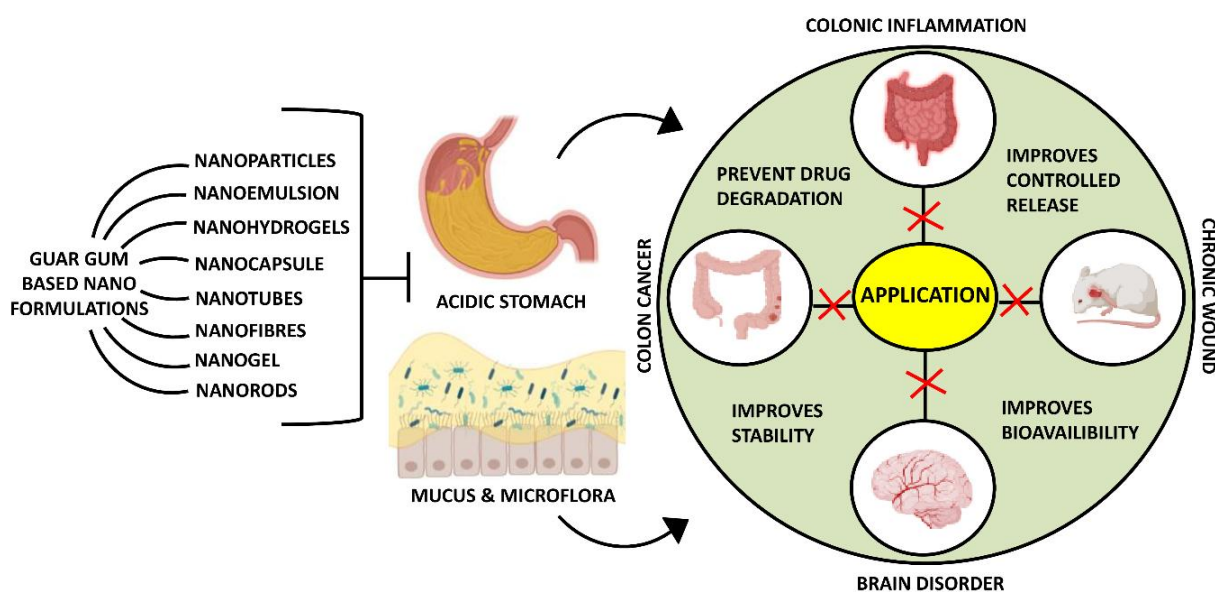


Figure 2.7: Biomedical applications of guar gum-based formulations for disease treatment through enhanced drug delivery.

Chapter 3

Hypothesis

The need for nutrition begins at fertilization, continues through life, and ends at death. A balance diet is composed of adequate amounts of essential nutrients, fats, carbohydrates, and proteins required by body to obtain energy, but any disturbance in a balanced diet leads to the development of diseases. For instance, nutrient deficiencies in humans can cause a variety of diseases, including protein energy malnutrition, anemia, pellagra, beriberi, and scurvy. Conversely, excessive nutrient consumption and their accumulation in adipocytes can results in obesity, which increases the risk of developing chronic diseases such as type 2 diabetes, inflammation, cardiovascular disease, cancer, and hypertension. Amongst all metabolic disorders, diabetes stands out as the most highly cited metabolic disorder, resulting from abnormalities in insulin secretion, insulin action, or both. The leading global diabetes organization, the International Diabetes Federation, reports that 583 million adults (aged 20-79 years) are currently living with diabetes worldwide, with this number projected to rise to 853 million by 2050. This escalation in the incidence of diabetes accentuates the need for the exploration of novel treatment strategies.

The approval of anti-diabetic drugs by the United States Food and Drug Administration has led to the widespread use of synthetic drugs globally for the treatment of diabetes. Some of the commonly used drugs include metformin, acarbose, glimepiride, sitagliptin, and repaglinide. While these drugs possess the potential to manage diabetes in clinical practice, concerns have arisen about their safety and effectiveness due to the limitations such as poor solubility, absorption, and low oral bioavailability, frequent administration with high dose, adverse side effects, including gastrointestinal side-effects, diarrhoea, nausea, gastric upset, abdominal pain. As aforementioned, while the use of synthetic drugs has reduced the global diabetic burden, qualms about their safety and efficacy persist due to these unwanted effects. This accentuates the need to look for an alternative that not only lower blood glucose levels but also reduce side effects and improve increase patients' quality of life.

The plant kingdom is the richest source of naturally occurring phenolic compounds, as there are about 8000 phenolic compounds in its distribution. Moreover, polysacchraides are long-chain carbohydrates compose of monosaccharide units linked together by glycosidic bonds. These polysaccharides can originate from plants, animals, algae, fungi, and microorganism. However, plant-based polysaccharides are the most widely used for enhancing drug delivery.

Guar gum is one such natural polysaccharide, extracted from the seeds of gaur plant (*Cyamopsis tetragonoloba*), and has been extensively used in drug delivery due to its ability to prolong drug release, enhance stability, enable colon-targeted drug release, and exhibit anti-diabetic properties. The abundance and diversity of plant-derived polysaccharides make them a valuable resource for developing novel drug delivery systems and therapeutic agents. This highlights the importance of using naturally occurring polysaccharides to enhance drug release and mitigate side-effects of established anti-diabetic medication, which often require frequent administration and results in various adverse effects. The encapsulation of such anti-diabetic medications in polymers like guar gum not only improves drug delivery by prolonging the drug release but also helps reduce the side effects associated with these drugs. Moreover, this approach could open new opportunities for researchers to discover the compatibility between drugs and polymers to predict their glucose lowering effects using *in vitro* and *in vivo* model of diabetes.

While many researchers are focused on exploring and validating the anti-diabetic effects of novel compounds, limited attention has been given to compounds that have already received regulatory approvals for their glucose lowering effects in treating diabetes. Since both acarbose and guar gum have received FDA approval, working with these compounds not only enhance their clinical application but also saves time, resources, and consumables needed for validating the anti-diabetic properties of novel compounds. This could potentially increase the likelihood of the developed formulation reaching clinical trials for human welfare.

Therefore, considering all these aspects, we propose to formulate an acarbose-encapsulated guar gum nanoformulation using spray-drying technology for improved anti-diabetic effects using *in vitro* and *in vivo* model of diabetes.

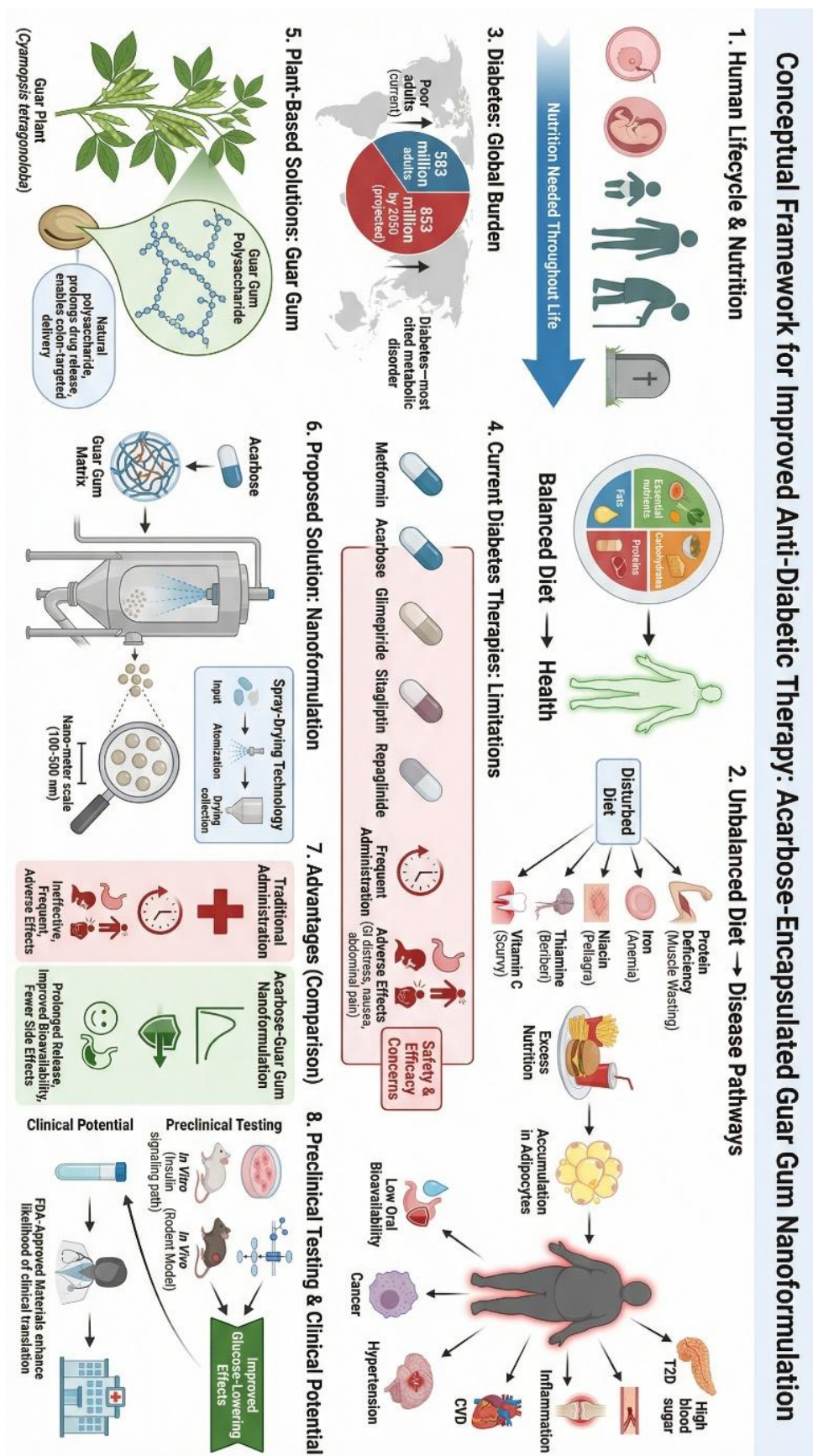


Figure 3.1: Conceptual framework of the proposed acarbose-encapsulated guar gum nanoformulation for improved anti-diabetic therapy.

Chapter 4

Aim and objectives

Diabetes mellitus is a major global health concern due to its associated complications and the limitations of current anti-diabetic drugs, such as poor solubility, low bioavailability, frequent dosing, and adverse side effects like diarrhoea. These challenges highlight the need for innovative therapeutic strategies that enhance drug efficacy and safety. Plant-based polysaccharides, particularly guar gum, offer promising potential for drug delivery due to their ability to prolong drug release, improve stability, target colon delivery, and exhibit anti-diabetic properties. The encapsulation of anti-diabetic drugs like acarbose in guar gum-based nanoformulation offers a promising strategy to address these challenges. This approach aims to enable sustained drug release, reduce adverse effects, and improve therapeutic efficacy with improved blood glucose profile.

Upon reviewing the pertinent literature, the following research gaps have been identified:

1. There is a need to develop a sustained-release formulation to minimize repetitive administration and reduce the gastrointestinal side effects of anti-diabetic drugs.
2. There is a need to formulate drug-encapsulated guar gum nanoformulation to achieve controlled drug release and improve blood glucose regulation.

To address these identified research gaps, the following objectives have been designed:

1. Development and characterization of drug encapsulated guar gum nanoformulation.
2. To evaluate the cytotoxic and anti-diabetic potential of drug encapsulated guar gum nanoformulation in *in vitro* diabetic models.
3. To evaluate the anti-diabetic potential of drug encapsulated guar gum nanoformulation in animal model of diabetes.

This study aims to provide a robust scientific foundation for utilizing guar gum-based nanoformulations as an advanced therapeutic strategy for diabetes management, addressing the limitations of existing treatment options.

Chapter 5

Material and Methodology

5.1. Chemicals and reagents

Unless specified otherwise, all chemicals and reagents employed in this study were of analytical grade. General laboratory reagents were procured from HiMedia Laboratories (India). Specific biochemical reagents, including ethylenediaminetetraacetic acid (EDTA; CAS: 60004), sodium orthovanadate (CAS: 13721396), sodium fluoride (CAS: 7681494), sodium pyrophosphate (CAS: 7722885), phenylmethylsulfonyl fluoride (PMSF; CAS: 329986), aprotinin (CAS: 9087701), sodium butyrate (CAS: 156547), thiobarbituric acid (CAS: 504176), nitroblue tetrazolium (NBT; CAS: 298839), hydroxylamine hydrochloride (CAS: 5470111), acetylthiocholine iodide (CAS: 1866155), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB; CAS: 69783), and hypoxanthine (CAS: 68940) were obtained from Sigma-Aldrich (USA). Acarbose was kindly supplied by Bayer Zydus Pharmaceuticals, Pune, India, under their student research welfare program.

5.2. Preliminary study

5.2.1 Assessment of polymer-acarbose binding via molecular docking

The interaction potential of acarbose (molecular weight 645.604 g/mol) with selected polymers was evaluated using molecular docking performed with AutoDock Vina (Trott and Olson, 2010). Three-dimensional (3D) structures of acarbose and the polymers were retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). The docking grid was configured to encompass the entire molecular structure, with grid points spaced at 0.375 Å. Coordinates of the grid origin (x, y, z) and dimensions were optimized to include all relevant atoms. Docking simulations were performed with an exhaustiveness parameter set to 20 to balance computational efficiency with predictive accuracy. The resulting docked complexes were ranked based on binding energy, and the most favorable polymer-acarbose interactions were visualized using Discovery Studio Visualizer (v.21.1.0.20298).

5.2.2 Assessment of stability of acarbose-polymer complexes via molecular dynamics simulation

Molecular dynamics (MD) simulations were performed using GROMACS 2024.2 to evaluate the stability of acarbose and its polymer complexes (Abraham et al., 2012). The simulation protocol consisted of three main steps. Initially, each acarbose–polymer complex was placed

in a cubic simulation box containing explicit SPC water molecules. Energy minimization of the solvated systems was conducted using the steepest descent algorithm for a maximum of 50,000 steps. Subsequently, MD simulations were carried out for 400 ns under isothermal-isobaric conditions (300 K, 1 atm). The stability of the complexes during the simulation was assessed by analyzing the root mean square deviation (RMSD) of atomic positions.

5.2.3 Preparation of samples

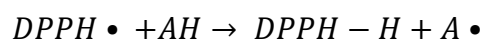
Aqueous solutions of acarbose and each selected polymer were prepared individually by dissolving in distilled water to obtain a final concentration of 1 mg/mL. Subsequently, mixtures of acarbose with the polymers were prepared in varying ratios of 100:0, 25:75, 75:25, and 50:50. The prepared samples were then stored at 4°C until further analysis (Oboh et al., 2016).

5.2.4 Assessment of DPPH radical scavenging activity

To evaluate the free radical scavenging ability of samples, the DPPH assay was performed.

Principle

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is based on the capacity of antioxidants to neutralize free radicals through hydrogen atom or electron donation. DPPH is a stable free radical that exhibits a deep violet color with strong absorbance at 517 nm. Upon reaction with an antioxidant, DPPH• is reduced to its non-radical form (DPPH-H), resulting in a decrease in absorbance and a color change from violet to yellow. The degree of discoloration provides a quantitative measure of the antioxidant's radical scavenging activity. The reduction reaction can be represented as follows:



where AH represents the antioxidant molecule and symbol (•) represents free radical.

Procedure

The antioxidant activity of acarbose, both individually and in combination with polymers, was evaluated using the DPPH assay as described by Sharma and Bhat (2009). Aqueous solutions of the test samples were prepared in the desired proportions and added to a reaction mixture containing 0.1 mM DPPH radicals. The mixture was stirred vigorously at 2,500 rpm for 1 min, followed by incubation in the dark for 30 min. The absorbance of the resulting solution was

measured at 517 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan).

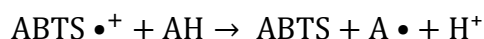
$$\% \text{ Radical Scavenging Activity} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100$$

5.2.5 Assessment of ABTS radical scavenging activity

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay was performed to evaluate the ability of antioxidants to quench ABTS^{•+} radicals, which represent both hydrophilic and lipophilic reactive species. This method provides a broad-spectrum assessment of radical quenching capacity.

Principle

The ABTS assay is based on the capacity of antioxidants to neutralize the ABTS^{•+} radical cation, a blue-green chromophore. ABTS^{•+} is generated by the oxidation of ABTS with an oxidizing agent, typically potassium persulfate. Antioxidants donate electrons or hydrogen atoms to reduce ABTS^{•+}, resulting in a decrease in absorbance. The extent of decolorization is directly proportional to the antioxidant capacity of the sample. This assay is applicable to both hydrophilic and lipophilic antioxidant systems. The reaction can be represented as:



Procedure

The ABTS^{•+} radical was generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate and incubating the solution in the dark at room temperature for 12-16 h. Subsequently, aqueous solutions of the test samples, prepared in various combinations, were added to the diluted ABTS^{•+} solution. The decrease in absorbance was measured at 734 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) (Obboh et al., 2016).

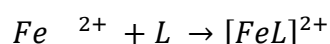
$$\% \text{ Radical Scavenging Activity} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100$$

5.2.6 Assessment of Fe²⁺ chelation activity

The Fe²⁺ chelation assay was performed to evaluate the ability of compounds to bind transition metal ions, particularly Fe²⁺, which can catalyze the formation of ROS via Fenton reactions. Metal-chelating activity reflects the preventive antioxidant potential of a compound.

Principle

The assay is based on the capacity of a compound to chelate ferrous ions (Fe^{2+}), thereby inhibiting the formation of the Fe^{2+} -ferrozine complex. Ferrozine forms a stable magenta-coloured complex with Fe^{2+} , which exhibits strong absorbance at 562 nm. In the presence of a metal-chelating agent, the formation of the Fe^{2+} -ferrozine complex is reduced, resulting in decreased absorbance. The extent of this decrease indicates the ferrous ion-chelating ability of the sample, reflecting its potential to prevent metal-catalyzed oxidative reactions:



Procedure

Aqueous samples at varying concentrations were mixed with 200 μL of buffer and 100 μL of freshly prepared Fe^{2+} solution, followed by incubation at room temperature for 10 min. Subsequently, 100 μL of a 1:10 phenanthroline solution was added, and the mixture was allowed to react for an additional 10 min to develop the color. Absorbance was measured at 510 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) (Obboh et al., 2016).

$$\% \text{ Chelation Activity} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100$$

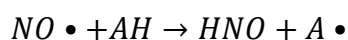
5.2.7 Assessment of nitric oxide radical scavenging activity

The nitric oxide ($\text{NO}\bullet$) radical scavenging assay was performed to evaluate the ability of compounds to neutralize nitric oxide radicals, which are implicated in inflammatory processes and oxidative stress. This assay reflects both anti-inflammatory and antioxidant potential.

Principle

The assay is based on the capacity of antioxidants to scavenge nitric oxide radicals. Sodium nitroprusside (SNP) in aqueous solution at physiological pH spontaneously generates nitric oxide, which reacts with oxygen to form nitrite ions. The nitrite ions are detected using Griess reagent, producing a pink-coloured chromophore. In the presence of an antioxidant, nitric oxide reacts preferentially with the antioxidant rather than oxygen, resulting in decreased nitrite

formation. The reduction in absorbance is directly proportional to the nitric oxide scavenging activity of the sample. The reaction can be represented as:



Procedure

To evaluate the nitric oxide scavenging activity of acarbose-polymer combinations, a reaction mixture was prepared containing 2 mL of 10 mM SNP and the test samples in phosphate-buffered saline (pH 7.4). The mixture was incubated at 25°C for 2.5 h. Subsequently, 0.5 mL of the reaction mixture was combined with 1 mL of sulfanilic acid (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for diazotization. Thereafter, 1 mL of N-(1-naphthyl) ethylenediamine (0.1% in water) was added, and the mixture was incubated for an additional 20 min at 25°C. The development of a pink color was monitored, and absorbance was recorded at 540 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) (Obboh et al., 2016).

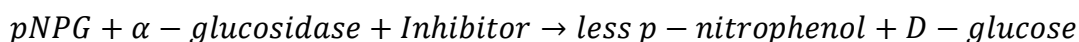
$$\% \text{ Nitric Oxide Scavenging Activity} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100$$

5.2.8 Assessment of α -glucosidase inhibition

The α -glucosidase inhibition assay was performed to evaluate the potential of compounds to delay carbohydrate digestion and glucose absorption, a mechanism relevant for controlling postprandial blood glucose levels in type 2 diabetes. Inhibition of α -glucosidase, the enzyme responsible for hydrolyzing complex carbohydrates into glucose, represents a therapeutic strategy for managing hyperglycemia.

Principle

The assay measures the ability of test compounds to suppress α -glucosidase activity. Normally, α -glucosidase hydrolyzes p-nitrophenyl- α -D-glucopyranoside (pNPG) to release p-nitrophenol, a yellow chromophore. The amount of p-nitrophenol formed is directly proportional to enzymatic activity and can be quantified spectrophotometrically. In the presence of an inhibitor, p-nitrophenol formation is reduced, leading to a decrease in absorbance. The extent of absorbance reduction reflects the α -glucosidase inhibitory potential of the sample:



Procedure

Aqueous solutions of the test compounds at varying proportions were mixed with 10 μL of α -glucosidase solution (1 U/mL) and 125 μL of 0.1 M phosphate buffer (pH 6.8), and incubated at 37°C for 20 min. The reaction was initiated by adding 20 μL of 1 M pNPG substrate, followed by incubation for an additional 30 min. The reaction was terminated with 50 μL of 0.1 N sodium carbonate, and absorbance was measured at 405 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) (Obboh et al., 2016).

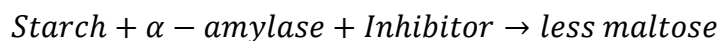
$$\% \alpha - \text{Glucosidase Inhibition} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100$$

5.2.9 Assessment of α -amylase inhibition

The α -amylase inhibition assay was performed to evaluate the ability of compounds to slow the enzymatic hydrolysis of dietary starch into glucose, thereby mitigating postprandial hyperglycemia. This is particularly relevant for the management of type 2 diabetes, where controlling blood glucose levels after meals is critical.

Principle

The assay is based on the inhibition of α -amylase, which catalyzes the hydrolysis of α -1,4-glycosidic bonds in starch to produce maltose and glucose. Starch serves as the substrate, and the amount of reducing sugars released by enzymatic activity is measured colorimetrically using 3,5-dinitrosalicylic acid (DNSA) reagent. DNSA reacts with reducing sugars to form a coloured complex. In the presence of an α -amylase inhibitor, less reducing sugar is produced, resulting in decreased absorbance. The reduction in absorbance reflects the inhibitory effect of the test compound:



Procedure

Freshly prepared aqueous solutions of the test compounds at varying proportions were mixed with 100 μL of α -amylase solution (1 U/mL) and 200 μL of 20 mM sodium phosphate buffer

(pH 6.9), followed by pre-incubation at 25°C for 10 min. The reaction was initiated by adding 200 µL of 1% starch solution (prepared in 20 mM sodium phosphate buffer, pH 6.9), and the mixtures were incubated at 25°C for 10 min. The reaction was terminated by adding 1 mL of DNSA reagent and placing the mixtures in a boiling water bath for 5 min. After cooling to room temperature, the solutions were diluted 1:5 with distilled water, and absorbance was measured at 540 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) (Obboh et al., 2016).

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

5.2.10 *In vivo* studies on *Drosophila melanogaster*

The *in vivo* effects of acarbose, alone and in combination with selected polymers, on antioxidant enzymes were evaluated using the wild-type strain *Drosophila melanogaster* (Oregon R⁺). This strain was kindly provided by Dr. Anurag Sharma (NITTE University, Mangalore, India) and was originally obtained from the Bloomington *Drosophila* Stock Center, Indiana, USA. The fly culture has been maintained at the Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Punjab, India, for the past seven years.

Drosophila melanogaster was chosen as an experimental model due to its highly conserved genetic, metabolic, and oxidative stress response pathways, which closely resemble those of humans. It offers a rapid, cost-effective, and ethically viable system for studying complex biological processes, including combinational therapies, and provides preliminary data to validate the efficacy and mechanistic insights of treatment strategies.

5.2.11 Culturing and grouping of *Drosophila* flies

Fly cultures were maintained on standard *Drosophila* medium (SDM) composed of maize powder (17 g), sulfur-free sugar (15 g), agar (1.5 g), and baker's yeast (6 g) per 360 mL of water. To prevent microbial contamination, 1 mL of propionic acid and 2-3 mL of sodium benzoate solution (1 g dissolved in 70-80% ethanol) were added to the medium. Cultures were maintained at 25 ± 1°C with 40-60% relative humidity, under a 12:12 h light/dark cycle, ensuring that light exposure did not exceed 14 h at 24 ± 1°C (Rahimi et al., 2022).

For experimental treatments, acarbose was combined with selected polymers, including pectin, β-cyclodextrin (β-CD), and guar gum, in varying ratios as described in the study.

Experimental design:

- **Group 1:** Normal control that received only Standard *Drosophila* Medium food
- **Group 2:** H₂O₂
- **Group 3:** H₂O₂ + Acarbose (100%)
- **Group 4:** H₂O₂ + Pectin (100%)
- **Group 5:** H₂O₂ + β -CD (100%)
- **Group 6:** H₂O₂ + Guar gum (100%)
- **Group 7:** H₂O₂ + 25% Acarbose + 75% Pectin
- **Group 8:** H₂O₂ + 75% Acarbose + 25% Pectin
- **Group 9:** H₂O₂ + 50% Acarbose + 50% Pectin
- **Group 10:** H₂O₂ + 25% Acarbose + 75% β -CD
- **Group 11:** H₂O₂ + 75% Acarbose + 25% β -CD
- **Group 12:** H₂O₂ + 50% Acarbose + 50% β -CD
- **Group 13:** H₂O₂ + 25% Acarbose + 75% Guar gum
- **Group 14:** H₂O₂ + 75% Acarbose + 25% Guar gum
- **Group 15:** H₂O₂ + 50% Acarbose + 50% Guar gum

5.2.12 Induction of oxidative stress and combined compound treatment

Oxidative stress was induced in *Drosophila melanogaster* (Oregon R⁺ strain) larvae by supplementing their standard diet with 1% H₂O₂ (1 mL). H₂O₂ was selected due to its well-established ability to diffuse across cell membranes and generate reactive oxygen species (ROS), thereby causing oxidative damage to cellular macromolecules.

The protective effects of acarbose, pectin, β -CD, and guar gum, both individually and in combination, were evaluated for their capacity to mitigate H₂O₂-induced oxidative stress. After exposure to H₂O₂, the test compounds were incorporated into the *Drosophila* diet in treatment vials, either alone or in the previously described combinations. Control vials contained Standard *Drosophila* Medium without any supplementation. Each treatment group consisted of 50 larvae transferred into vials containing 10 mL of standard medium supplemented with the respective compounds (Rahimi et al., 2022; Omoboyowa et al., 2022).

5.2.13 Preparation of larval homogenate for analysis

After 48 hours, at the onset of pupation, larvae from each treatment vial were collected for protein extraction. Larvae were homogenized in a low-salt buffer containing 10 mM Tris-HCl

(pH 7.4), 150 mM EDTA, 1 mM sodium orthovanadate, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride, 10 mg/mL aprotinin, 1 mM sodium butyrate, and 0.05% NP-40. The homogenate was centrifuged at $10,000 \times g$ for 10 min at 4°C, and the supernatant, containing soluble proteins, was collected for further analysis, while the pellet was discarded. Protein samples were kept on ice and stored at -4°C until enzymatic assays were performed (Rahimi et al., 2022).

5.2.14 Assessment of protein content

Protein concentration in the larval homogenate supernatant was measured using the Lowry method, with bovine serum albumin (BSA) as the standard (Lowry et al., 1951). This method provides a reliable estimation of total protein levels and serves as a basis for subsequent enzymatic activity assays.

Principle

The Lowry method relies on the biuret reaction, wherein proteins in an alkaline solution form complexes with copper ions (Cu^{2+}), reducing them to Cu^+ . The resulting copper-protein complex reacts with the Folin-Ciocalteu reagent, generating a blue chromophore. The overall process involves two main stages: (i) protein-copper complex formation and (ii) reaction of this complex with the Folin-Ciocalteu reagent, leading to a measurable color change. Protein concentration is quantified by comparing the absorbance of the sample to a standard curve prepared with a known concentration of BSA.

Procedure

Lowry's reagent was prepared by mixing Reagent A (2% sodium carbonate in 0.1 N NaOH), Reagent B (1% CuSO_4 in double-distilled water), and Reagent C (2% sodium potassium tartrate in double-distilled water) in a 49:0.5:0.5 (v/v) ratio. A stock protein standard was prepared by dissolving 20 mg of BSA in 1 mL of double-distilled water. For the assay, blank, standard, and test tubes were prepared by adding 1 mL DDW (blank), 0.1 mL standard, or 0.1 mL test sample, respectively, along with 5 mL of Lowry's reagent. Tubes were incubated at 37 °C for 15 min, followed by the addition of 0.5 mL Folin-Ciocalteu reagent (diluted 1:1 with DDW). After a further 30 min incubation at 37 °C, absorbance was measured at 670 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Shimadzu, Kyoto, Japan).

5.2.15 Assessment of LPO damage

The LPO assay was performed to evaluate oxidative damage to lipids, particularly in cellular membranes. By quantifying LPO products such as MDA, this assay provides insight into the antioxidant defense capacity of a sample and the effectiveness of antioxidant interventions.

Principle

LPO occurs when ROS attack unsaturated fatty acids in cellular membranes, generating MDA as one of the end products. In this assay, MDA reacts with thiobarbituric acid (TBA) under acidic conditions to form a pink-coloured complex. The absorbance of this complex, measured spectrophotometrically at 532 nm, is directly proportional to the MDA concentration and, hence, the extent of LPO. This method is commonly known as the TBARS (Thiobarbituric Acid Reactive Substances) assay.

Procedure

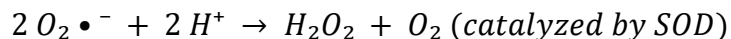
A modified version of the method described by Iqbal et al. (1996) was employed. The reaction mixture consisted of 1000 μL of 150 mM Tris-HCl buffer (pH 7.1), 100 μL of sample lysate, 100 μL of 1 mM ferrous sulfate, and 100 μL of 1.5 mM ascorbic acid, for a final volume of 1300 μL . The mixture was incubated at 37°C for 60 min. To terminate the reaction, 1000 μL of 10% trichloroacetic acid was added, followed by 1 mL of 10% TBA. Tubes were tightly capped and heated in a boiling water bath for 15 min. After heating, samples were rapidly cooled on ice and centrifuged at $3000 \times g$ for 10 min. The absorbance of the supernatant was recorded at 535 nm against a reagent blank using a UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). MDA concentration was calculated using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and expressed as $\mu\text{mol MDA}/\text{min}/\text{mg tissue}$ at 37°C.

5.2.16 Assessment of SOD activity

The SOD activity assay was performed to evaluate the activity of SOD, a key antioxidant enzyme responsible for protecting cells from ROS, particularly the superoxide anion ($\text{O}_2^{\bullet-}$). SOD catalyzes the dismutation of superoxide into H_2O_2 and O_2 , thereby mitigating oxidative stress. Measurement of SOD activity provides insight into the antioxidant defense status of cells or tissues and is relevant in conditions such as inflammation, neurodegeneration, cancer, and diabetes.

Principle

The assay is based on the catalytic dismutation of $O_2^{\bullet -}$ by SOD into H_2O_2 and O_2 :



In this method, superoxide radicals generated by hydroxylamine hydrochloride reduce NBT to a coloured formazan product. The presence of SOD inhibits nitroblue tetrazolium (NBT) reduction, resulting in a decrease in color formation. SOD activity is inversely proportional to the extent of NBT reduction.

Procedure

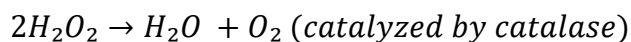
SOD activity was estimated according to Beyer and Fridovich (Beyer et al., 1987). The reaction mixture comprised 500 μ L of NBT (96 μ M), 100 μ L of Triton X-100 (0.6%), and 1.3 mL of sodium carbonate (50 mM, pH 10.0). The reaction was initiated by adding 100 μ L of hydroxylamine hydrochloride (20 mM, pH 6.0), followed by 70 μ L of the lysate. The increase in absorbance due to NBT reduction was measured at 540 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Shimadzu, Kyoto, Japan) over 5 minutes, with readings at 60-second intervals. One unit of SOD activity was defined as the enzyme quantity required to inhibit 50% of NBT reduction.

5.2.17 Assessment of catalase activity

The catalase activity assay was performed to evaluate the activity of catalase, a key antioxidant enzyme that protects cells from oxidative damage by decomposing H_2O_2 . Catalase is integral to the antioxidant defense system, preventing the accumulation of H_2O_2 and thereby mitigating oxidative stress.

Principle

The assay is based on the ability of catalase to decompose H_2O_2 into water and oxygen:



The decomposition of H_2O_2 is monitored either by measuring the rate of oxygen evolution or by observing the decrease in absorbance, as H_2O_2 absorbs UV light at the assay wavelength.

Procedure

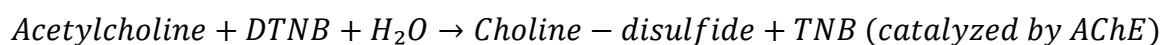
Catalase activity was determined using the method of Chance and Maehly (1954). The reaction mixture contained 3000 μL of phosphate buffer (50 mM, pH 5), 25 μL of sample, and H_2O_2 (5.9 mM). The decrease in absorbance at 240 nm was recorded at 30-second intervals for 2 minutes using a UV-visible spectrophotometer (Shimadzu UV-1800, Shimadzu, Kyoto, Japan). Catalase activity was calculated using the molar extinction coefficient and expressed as micromoles of H_2O_2 decomposed per minute per milligram of protein.

5.2.18 Assessment of AChE activity

The AChE activity assay was performed to evaluate cholinergic neurotransmission. Elevated AChE activity is associated with acetylcholine depletion, contributing to cognitive decline in Alzheimer's disease, while in diabetes, altered AChE activity can impact insulin secretion and cognitive function. This assay allows assessment of cholinergic dysfunction and evaluation of potential therapeutic interventions aimed at restoring AChE balance.

Principle

The assay measures the hydrolysis of acetylcholine by AChE into choline and acetic acid. The released choline reacts in a coupled reaction with choline oxidase to produce H_2O_2 , which is subsequently quantified colorimetrically using a chromogenic reagent. The color intensity is directly proportional to AChE activity. The enzymatic reaction can also be monitored by measuring the absorbance change at a specific wavelength, reflecting the amount of acetylcholine hydrolyzed:



Procedure

AChE activity was assessed by measuring absorbance changes at 405 nm (Rahimi et al., 2022). The substrate, acetylthiocholine, is hydrolyzed by AChE to produce thiocholine and acetate. Thiocholine then reduces Dithiobis-Nitrobenzoic Acid (DTNB), releasing nitrobenzoate, which can be quantified spectrophotometrically. The reaction mixture consisted of 135 μL of distilled water, 20 μL of 100 mM potassium phosphate buffer (pH 7.4), 20 μL of 8 mM acetylthiocholine, and 20 μL of 10 mM DTNB. Finally, 5 μL of the lysate was added, and the

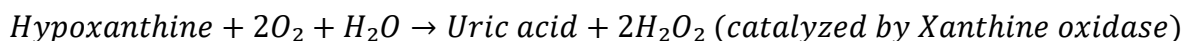
change in absorbance per minute was recorded at 405 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Shimadzu, Kyoto, Japan).

5.2.19 Assessment of Xanthine oxidase activity

The xanthine oxidase activity assay was performed to evaluate its contribution to oxidative stress, which is implicated in neurodegenerative disorders and diabetes. Xanthine oxidase generates ROS that induce oxidative damage; in neurodegenerative diseases such as Alzheimer's, xanthine oxidase-derived ROS exacerbate neuroinflammation and cognitive decline. In diabetes, elevated xanthine oxidase activity promotes insulin resistance and complications like diabetic neuropathy. This assay provides insight into oxidative stress levels and the potential efficacy of therapeutic interventions targeting xanthine oxidase.

Principle

The assay is based on the ability of xanthine oxidase to catalyze the oxidation of xanthine (or hypoxanthine) to uric acid, simultaneously producing ROS, including $O_2^{\bullet-}$ and H_2O_2 . Uric acid formation can be quantified spectrophotometrically, as uric acid absorbs light at 290 nm. The increase in absorbance reflects xanthine oxidase activity and indirectly indicates ROS production:



Procedure

Xanthine oxidase activity was determined by measuring the rate of urate formation from hypoxanthine. The reaction mixture consisted of 1 mL of enzyme lysate, 0.1 mL of hypoxanthine, and 2 mL of phosphate buffer (pH 7.5). Absorbance was recorded at 290 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Shimadzu, Kyoto, Japan). One unit of xanthine oxidase activity was defined as the production of 1 μmol of urate per minute at 25°C. The molar absorptivity of uric acid was considered as $1.22 \times 10^4 \text{ cm}^{-1}$ (Rahimi et al., 2022).

5.3 Formulation development and characterization study

5.3.1 Preparation and optimization of formulations

Solid dispersion-based nanoformulations of acarbose and guar gum were developed in three different drug-to-polymer ratios: 1:1, 1:2, and 1:3 (Ho et al., 1996; Sinha et al., 2010). The formulations were coded as AEGN-1 (1:1), AEGN-2 (1:2), and AEGN-3 (1:3).

In the initial preparations, 3 g of acarbose was combined with 3 g, 6 g, and 9 g of guar gum for AEGN-1, AEGN-2, and AEGN-3, respectively, and each mixture was dissolved in 500 mL of Milli-Q water. The solutions were stirred overnight using a magnetic stirrer to ensure complete dissolution and uniform dispersion. During spray drying, formulations with higher polymer content (AEGN-2 and AEGN-3) exhibited processing challenges due to increased viscosity, which compromised the spray-drying efficiency. To overcome this, the compositions were optimized in subsequent preparations. For the optimized formulations, AEGN-1 consisted of 3 g acarbose and 3 g guar gum, whereas AEGN-2 and AEGN-3 contained 2 g acarbose with 4 g and 6 g guar gum, respectively, in 1000 mL of Milli-Q water. All mixtures were stirred overnight to achieve homogeneity prior to spray drying. This adjustment successfully reduced viscosity and enhanced processability during spray drying.

Nano spray drying of the aqueous dispersions was performed using a Techno Spray dryer (SPD-P-111-21/22-13, Techno Search Process & Systems, Thane, India) to obtain stable nanoformulations. Atomization was achieved via vibrating mesh technology, and particles were collected electrostatically. Spray-drying parameters were standardized as follows: nozzle diameters of 5.5 and 7.0 μm , inlet and outlet temperatures of 120 $^{\circ}\text{C}$ and 90 $^{\circ}\text{C}$, feed rate of 0.4 mL/min, airflow of 140-150 L/min, atomization pressure of 25 psi, and spray rate of 75%. The resulting powders were collected with a scraper and stored in a desiccator for further evaluation.

In parallel, physical mixtures (PM) were prepared by blending the drug and polymer in identical ratios. For 1:1 (PM1), 1:2 (PM2), and 1:3 (PM3), 500 mg of acarbose was mixed with 500 mg, 1000 mg, and 1500 mg of guar gum, respectively. The components were homogenized using a mortar and pestle to obtain fine powders, which were stored in a desiccator for subsequent analysis.

5.3.2 Particle size and zeta potential analysis

The spray-dried nanoformulations were dispersed in Milli-Q water at a concentration of 1 mg/mL and sonicated for 30-35 minutes to achieve uniform dispersion. The suspension was subsequently filtered through a 0.45 μm syringe filter to remove aggregates and transferred to a clean cuvette. Particle size, polydispersity index (PDI), and zeta potential were determined using a Malvern Zetasizer Nano ZS90 at 25 $^{\circ}\text{C}$ (Sharma et al., 2013).

5.3.3 SEM analysis for surface morphology

The surface morphology of pure acarbose, guar gum, and the developed solid dispersions was examined using a scanning electron microscope (S-3400 N, Hitachi, Tokyo, Japan). Samples were mounted on SEM stubs using double-sided carbon adhesive tape and coated with a thin layer of gold using an ion sputter coater (E-1010, Hitachi) for 15 s per cycle over 3-4 cycles to enhance electrical conductivity. SEM images were acquired at an accelerating voltage of 15 kV with a working distance of 10 mm (Gupta et al., 2014).

5.3.4 Crystallographic analysis by pXRD

pXRD was performed to investigate the crystalline or amorphous nature of the samples. Diffraction patterns were recorded using a SmartLab 9 kW rotating anode diffractometer (Rigaku Corporation, Tokyo, Japan) with Cu-K α radiation, operating at 40 kV and 30 mA. Samples were scanned over a 2θ range of 10° - 60° at a scan rate of $10^\circ/\text{min}$. Diffractograms were analyzed to identify characteristic peaks and assess any reduction in crystallinity or transition to the amorphous state following spray drying (Khan et al., 2023).

5.3.5 Analysis of functional groups by FTIR

FTIR spectroscopy was performed to confirm the presence of functional groups and to evaluate potential chemical interactions or incompatibilities within the developed nanoformulations. Infrared spectra were recorded using an FTIR spectrophotometer (Perkin Elmer, Waltham, MA, USA) equipped with Spectrum ES software (version 10.5.3) over a spectral range of 400-4000 cm^{-1} . Solid samples were prepared as KBr pellets by mixing the sample with potassium bromide in an approximate mass ratio of 10:1 and compressing the mixture to form transparent discs suitable for analysis (Khan et al., 2023).

5.3.6 Determination of drug content

The drug content of developed formulations was quantified using UV-visible spectrophotometry (Genesys 180, Thermo Scientific, USA). A standard calibration curve was prepared by dissolving 10 mg of pure acarbose in 10 mL HPLC-grade Milli-Q water, followed by vortexing to ensure complete dissolution. Spray-dried samples were similarly dissolved and analyzed under the same conditions (Masaeli et al., 2016). The acarbose content in each formulation was determined from the standard curve, and percentage drug content was calculated using the following equation:

$$\% \text{ Drug content} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

5.3.7 Determination of drug entrapment efficiency

Drug entrapment efficiency of the nanoformulations was determined by quantifying the amount of untrapped (free) drug in the supernatant. Briefly, 10 mg of each formulation was dissolved in 10 mL HPLC-grade Milli-Q water and vortexed to ensure complete dispersion. The suspension was centrifuged at 3000 rpm for 5 min to separate the untrapped drug. The concentration of free acarbose in the supernatant was measured using UV-visible spectrophotometry (Genesys 180, Thermo Scientific, USA). Drug entrapment efficiency (%) was calculated as:

$$\% \text{ Drug Entrapment Efficiency} = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

5.3.8 *In vitro* release study

The *in vitro* release of acarbose from the developed solid dispersions was assessed using a dialysis membrane diffusion method (14 kDa molecular weight) (Kumar & Sinha, 2012). The dialysis membrane was pre-soaked in PBS (pH 6.8) for 24 h. Pure acarbose or the solid dispersion (equivalent to 5 mg drug) was dispersed in 5 mL PBS and transferred into the pre-soaked dialysis bag. The bag was then immersed in 100 mL PBS at 37 ± 0.5 °C and stirred at 150 rpm. At predetermined time intervals, 2 mL aliquots were withdrawn from the external medium and replaced with an equal volume of fresh PBS. Aliquots were filtered through a 0.22 µm membrane filter and analyzed using UV-visible spectrophotometry (Genesys 180, Thermo Scientific, USA) at 210 nm. A guar gum control solution (without drug) was analyzed under the same conditions and showed negligible absorbance, confirming method specificity.

5.3.9 Stability study

The stability of the spray-dried formulations was evaluated through an accelerated stability study in accordance with ICH Q1A (R2) guidelines. Formulations were sealed in airtight glass vials and stored in a stability chamber maintained at 40 ± 2 °C and $75 \pm 5\%$ relative humidity (RH). Samples were withdrawn at 0, 1, 3, and 6 months and analyzed for particle size, PDI, zeta potential, drug content, drug entrapment efficiency, and *in vitro* drug release profile. All analyses were performed in triplicate to ensure reproducibility and to detect any significant changes in the physicochemical characteristics of the formulations over time.

5.4 *In vitro* biological activity

5.4.1 Sample preparation for assessment of non-enzymatic antioxidant properties of AEGNs

Aqueous stock solutions of all compounds and formulations were prepared by dissolving them in distilled water to achieve a concentration of 1 mg/mL. From this stock, working solutions at concentrations of 20, 40, 60, 80, and 100 µg/mL were prepared through appropriate serial dilutions. These working concentrations were subsequently used to assess the non-enzymatic antioxidant activities of the formulations (Sharma et al., 2009; Oboh et al., 2016).

5.4.2 DPPH radical scavenging activity

The DPPH radical scavenging activity of the AEGNs was evaluated at varying concentrations following the protocol described by Sharma et al. (2009). The radical scavenging capacity was quantified and expressed as IC₅₀ values (µg/mL), representing the concentration of AEGNs required to neutralize 50% of DPPH radicals.

5.4.3 ABTS radical scavenging activity

The ABTS radical scavenging activity was assessed for all AEGNs formulations at different concentrations, according to the method described by Oboh et al. (2016). The results are expressed as IC₅₀ values (µg/mL), indicating the concentration necessary to scavenge 50% of ABTS radicals.

5.4.4 Fe²⁺ chelation assay

The metal chelation potential of AEGNs was evaluated using Fe²⁺ ions at varying concentrations, following the previously described protocol (Oboh et al., 2016). The chelating activity is expressed as IC₅₀ values (µg/mL), representing the concentration required to chelate 50% of the ferrous ions.

5.4.5 Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of the AEGNs was assessed at multiple concentrations following the methodology outlined by Oboh et al. (2016). The results are expressed as IC₅₀ values (µg/mL), corresponding to the concentration at which 50% of nitric oxide radicals are neutralized.

5.4.6 α -Glucosidase inhibitory assay

The α -glucosidase inhibitory activity of AEGNs was evaluated using concentrations of 20, 40, 60, 80, and 100 $\mu\text{g/mL}$, as per the previously established protocol (Oboh et al., 2016). The inhibitory activity is represented as IC_{50} ($\mu\text{g/mL}$), indicating the concentration required to inhibit 50% of α -glucosidase activity.

5.4.7 α -Amylase inhibitory assay

The α -amylase inhibitory potential of AEGNs was assessed at the same concentrations and using the same methodology described for the α -glucosidase assay (Oboh et al., 2016). The results are expressed as IC_{50} ($\mu\text{g/mL}$), reflecting the concentration necessary to inhibit 50% of α -amylase activity.

5.4.8 Culturing and Maintenance of cell lines

Prior to evaluating the effects of AEGNs on HFD-fed, STZ-induced diabetic animal models, the required cell lines were procured from the National Centre for Cell Science, Pune, India. The cell lines included BHK-21 (Baby Hamster Kidney), L929 (Mouse fibroblast cells), L6 (Rat skeletal muscle cells), 3T3 (Mouse embryonic fibroblast cells), CaCo2 (Human colorectal adenocarcinoma cells), and HepG2 (Human hepatocellular carcinoma cells). All cell lines were maintained in the Animal Tissue Culture Laboratory, Department of Biochemistry, School of Bioengineering and Biosciences, Lovely Professional University, India.

Cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% antibiotic solution (penicillin, 100 IU/mL, and streptomycin, 100 $\mu\text{g/mL}$). Cultures were maintained under standard conditions at 37°C in a humidified atmosphere containing 5% carbon dioxide (Botta et al., 2014).

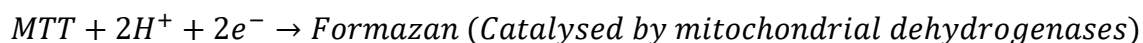
5.4.9 Preclinical cytotoxicity assessment of AEGNs using MTT assay

The MTT assay was employed to evaluate the cytotoxicity and cell viability of AEGNs by measuring cellular metabolic activity. This assay serves as a critical preliminary screening tool to determine the safety of test compounds before *in vivo* studies, aiding in appropriate dose selection and minimizing unnecessary animal usage.

Principle

The MTT assay is based on the reduction of the yellow, water-soluble tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into insoluble purple formazan crystals by metabolically active cells. This conversion is catalyzed primarily by mitochondrial

enzymes, including succinate dehydrogenase. Non-viable cells lack this enzymatic activity and therefore do not generate formazan. The resulting formazan crystals are dissolved in an organic solvent such as dimethyl sulfoxide, and the absorbance of the resulting solution is measured spectrophotometrically, typically at 570 nm. The absorbance intensity correlates directly with the number of viable cells, allowing quantitative assessment of cytotoxicity and cell proliferation.



Procedure

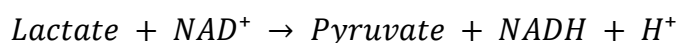
To assess cytotoxicity, BHK-21, L929, L6, 3T3, CaCo2, and HepG2 cells were seeded at a density of 1×10^4 cells per well in 96-well plates and incubated at 37°C in a 5% CO₂ atmosphere for 24 hours to allow cell adhesion. Cells were then treated with graded concentrations of acarbose, guar gum, and the AEGN formulations (AEGN-1, AEGN-2, and AEGN-3) at 6.25, 12.5, 25, 50, and 100 µg/mL in triplicate and incubated overnight at 37°C. Following treatment, 20 µL of MTT solution was added to each well, and the plates were incubated for 4 hours at 37°C. The medium was then discarded, and the resulting formazan crystals were dissolved in 100 µL of dimethyl sulfoxide. Plates were incubated for 30 minutes to ensure complete dissolution, and absorbance was measured at 540 nm using an ELISA plate reader (MPM 6, Bio-Rad) (Öztürk et al., 2023). All experiments were conducted in triplicate. Cell viability was calculated, and the half-maximal inhibitory concentration (IC₅₀), representing the concentration required to inhibit 50% of cell proliferation, was determined and expressed in µg/mL. Results are presented as mean ± SEM.

5.4.10 Assessment of membrane damage using LDH assay

The LDH assay was employed to evaluate cell membrane integrity as an indicator of cytotoxicity. LDH is a stable cytoplasmic enzyme present in most eukaryotic cells and is released into the extracellular environment upon membrane damage caused by cellular injury or stress. Quantification of extracellular lactate dehydrogenase activity provides a reliable measure of cell death, particularly necrosis, and is widely used in drug screening, toxicology, and assessment of cytoprotective or cytotoxic effects of compounds *in vitro* and *ex vivo*.

Principle

The assay is based on the enzymatic oxidation of lactate to pyruvate, catalyzed by lactate dehydrogenase in the presence of the coenzyme NAD⁺ (nicotinamide adenine dinucleotide). During this reaction, lactate dehydrogenase converts lactate into pyruvate while simultaneously reducing NAD⁺ to NADH. The generated NADH is subsequently coupled to a colorimetric reaction, in which NADH reduces a tetrazolium salt via an intermediate electron acceptor to form a coloured formazan product. The intensity of the color, measured spectrophotometrically, is directly proportional to lactate dehydrogenase activity and, therefore, to the extent of cellular damage or lysis.



Procedure

HepG2 and L6 cells were used to assess membrane damage. Cells were seeded in 96-well plates at a density of 1×10^4 cells per well and incubated at 37°C in a 5% CO₂ atmosphere until reaching approximately 80% confluency. Following this, the following treatments were applied in triplicate: 10 µL of lysis solution to LDH control wells, 10 µL of test compounds at graded concentrations (6.25, 12.5, 25, 50, and 100 µg/mL) to experimental wells, 10 µL of solvent to untreated control wells, and 10 µL of Dulbecco's phosphate-buffered saline to background control wells. Dose selection was guided by IC₅₀ values determined from the MTT assay.

The plates were incubated at 37°C for 10 minutes, followed by the addition of 50 µL of LDH reagent to each well. The plates were further incubated at room temperature for 30 minutes. Subsequently, 50 µL of Stop solution was added to terminate the reaction, and the absorbance was measured at 580 nm using a microplate reader (MPM 6, Bio-Rad). The percentage of lactate dehydrogenase release, indicative of cell membrane damage, was calculated according to the manufacturer's instructions using the LDH assay kit (HiMedia, India) (Kumar et al., 2018).

$$\% \text{ LDH release} = \frac{(\text{Maximum LDH control} - \text{Background control})}{(\text{Experimental control} - \text{Background control})} \times 100$$

5.4.11. *In vitro* assessment of blood compatibility using hemolysis assay

The hemolysis assay was employed to evaluate the blood compatibility of the developed formulations and their potential to induce red blood cell (RBC) damage. Hemolysis, defined as the disruption of the erythrocyte membrane leading to hemoglobin release into the extracellular medium, serves as a primary indicator of hemotoxicity. Excessive hemolysis may trigger

adverse biological responses, making this assay essential for preliminary safety assessment of pharmaceutical formulations, biomaterials, and nanocarriers.

Principle

The assay is based on the measurement of hemoglobin released from RBCs upon membrane disruption. Exposure of RBCs to a test compound that compromises membrane integrity results in the leakage of intracellular hemoglobin into the surrounding medium. The released hemoglobin exhibits strong absorbance at 540 nm, which can be quantified spectrophotometrically to assess the extent of hemolysis.

Procedure

Fresh whole blood (4 mL) was collected from a healthy rat and transferred into an EDTA-coated tube to prevent coagulation (Mehta et al., 2025). RBCs were isolated by centrifugation at 1500 rpm for 10 minutes at 4 °C, and the pellet was washed repeatedly with ice-cold 0.9% sodium chloride until the supernatant was clear. The purified RBCs were resuspended in ice-cold 0.9% sodium chloride to prepare a uniform erythrocyte suspension.

Test compounds, including acarbose, guar gum, and the spray-dried solid dispersions AEGN-1, AEGN-2, and AEGN-3, were individually incubated with the RBC suspension at 37 °C for 1 hour with gentle continuous mixing. Saline (0.9% NaCl) served as a negative control, and 0.1% Triton X-100 in 0.9% NaCl was used as a positive control. Following incubation, samples were centrifuged under the same conditions to separate intact RBCs from released hemoglobin. The supernatant absorbance was measured at 540 nm using an ELISA microplate reader (MPM 6, Bio-Rad).

The percentage of hemolysis was calculated using the following equation:

$$\% \text{ Haemolysis} = \frac{\{Absorbance (Sample) - Absorbance (Negative control)\}}{\{Absorbance (Triton) - Absorbance (Negative control)\}} \times 100$$

5.4.12 *In vitro* induction of diabetes in cells cultured in 6-well plate

Hyperglycemic conditions were simulated *in vitro* to mimic diabetic states and evaluate oxidative stress-related cellular responses. Cells (BHK-21, L6, HepG2, and 3T3) were cultured in high-glucose medium (30 mM) to induce diabetes-like conditions. Elevated extracellular glucose promotes oxidative stress through increased generation of ROS, which can damage cellular macromolecules, including lipids, proteins, and DNA.

Parallel cultures were maintained in low-glucose medium (5 mM) to represent normoglycemic conditions, providing a controlled baseline for comparison (Weil et al., 2009; Sato et al., 2002). Cells were treated with graded concentrations of test compounds, selected based on IC₅₀ values obtained from the MTT assay, ensuring the doses were within non-cytotoxic ranges. Following incubation under hyperglycemic or normoglycemic conditions, antioxidant enzyme activities were assessed to model cellular redox status. BHK-21 and 3T3 fibroblasts were used to evaluate general cellular oxidative responses, HepG2 hepatocytes to assess hepatic oxidative modulation, and L6 myoblasts to represent skeletal muscle, a primary tissue affected in diabetes.

All experiments were conducted in triplicate (n = 3 independent experiments), with three technical replicates per condition, following established protocols to enable robust detection of glucose- and concentration-dependent effects. This *in vitro* model provides a platform to evaluate the protective effects of AEGNs against diabetes-associated oxidative stress in multiple cellular contexts.

Experimental design:

Glucose Conditions:

- Low glucose (5 mM)
- High glucose (30 mM)

Treatment Groups (per glucose condition):

- Control (5 mM and 30 mM)
- Acarbose (50, 100 µg/mL) in both 5 mM and 30 mM
- Guar gum (50, 100 µg/mL) in both 5 mM and 30 mM
- AEGN-1 (50, 100 µg/mL) in both 5 mM and 30 mM
- AEGN-2 (50, 100 µg/mL) in both 5 mM and 30 mM
- AEGN-3 (50, 100 µg/mL) in both 5 mM and 30 mM

5.4.13 Cell lysis and preparation of cell lysate for protein array (6-well format)

BHK-21, L6, HepG2, and 3T3 cells cultured under low- and high-glucose conditions were harvested for protein analysis. Cell lysis was performed using a low-salt buffer (LSB)

containing 10 mM Tris-HCl (pH 7.4) to maintain physiological pH, 150 mM EDTA to chelate divalent cations and inhibit metal-dependent enzymes, 10 mM sodium fluoride as a phosphatase inhibitor, 1 mM PMSF to prevent proteolytic degradation, and 0.05% NP-40, a non-ionic detergent, to solubilize cellular membranes.

Following experimental treatments, wells were rinsed with ice-cold phosphate-buffered saline (PBS) to remove residual media and serum. Lysis buffer was then added to each well, and cells were mechanically detached using a cell scraper. The resulting lysates were collected in pre-chilled microcentrifuge tubes and centrifuged at 1700 rpm for 7 minutes at 4 °C. The supernatant, containing soluble proteins, was carefully collected for subsequent protein array analysis, while the pellet was discarded (Kushwaha et al., 2024).

5.4.14 Preparation of tissue homogenate and Post-Nuclear Supernatant (PNS) and Post-Mitochondrial Supernatant (PMS) fractions

Homogenates were prepared from BHK-21, L6, HepG2, and 3T3 cells cultured under low- and high-glucose conditions. The PNS was used for the assessment of GST activity, while the PMS was employed for measuring catalase, SOD activities, and LPO markers, including MDA. Since catalase and SOD are primarily cytosolic enzymes, their activity is accurately assessed in the PMS fraction rather than in whole-cell homogenate, which contains intact organelles that may interfere with enzymatic measurements. Similarly, MDA reflects cytoplasmic LPO and is best measured in PMS.

A homogenization buffer was prepared by mixing 0.1 M EDTA and 0.1 M tris chloride, adjusting the pH to 7.4, and adding β -mercaptoethanol immediately prior to homogenization. Cells were homogenized using a motorized Teflon pestle at 3000 rpm within a Dounce homogenizer, and the homogenization tube was maintained in an ice-filled beaker to prevent protein denaturation (Kushwaha et al., 2024). The resulting mixture was filtered through cotton gauze and stored in labeled vials at -18 °C until further use. A 10% homogenate was prepared (1 g of cells in 9 mL buffer) as the stock, and working homogenates were obtained by appropriate dilution with buffer.

To isolate the PNS and PMS fractions, the homogenate was centrifuged at 10,000 rpm for 5 minutes at 4 °C using a C-24 REMI cold centrifuge. The nuclear pellet was discarded, and the supernatant (PNS), containing nuclear and mitochondrial components, was collected and stored at -18 °C. The PNS was further centrifuged at 10,000 g for 10 minutes at 4 °C to separate the mitochondrial pellet. The resulting supernatant, the PMS, which is enriched in cytosolic and

microsomal components, was collected and stored at -18 °C for subsequent enzymatic and oxidative stress assays (Kushwaha et al., 2024).

5.4.15 Estimation of protein content using Lowry's method

Total protein concentration in lysates prepared from BHK-21, L6, HepG2, and 3T3 cells cultured under low- and high-glucose conditions was determined using the method described by Lowry et al. (1951). A standard calibration curve was generated using bovine serum albumin at concentrations of 20, 40, 60, 80, and 100 µg/mL. Protein concentrations of experimental samples were calculated from the standard curve and expressed as µg/mg protein.

5.4.16 Assessment of LPO in cell lysates

To evaluate the effect of AEGNs on lipid peroxidation, cell lysates prepared from BHK-21, L6, HepG2, and 3T3 cells cultured under normoglycemic and hyperglycemic conditions were analyzed for MDA levels. MDA, a secondary product of lipid peroxidation, serves as a reliable marker of oxidative membrane damage. MDA levels were determined according to the method described by Iqbal et al. (1996), allowing comparison of oxidative injury between normal and high-glucose conditions.

5.4.17 Assessment of SOD Activity in cell lysates

SOD activity was measured in cell lysates prepared from BHK-21, L6, HepG2, and 3T3 cells following treatment with various compounds. The assay was performed according to the method described by Beyer et al. (1987).

5.4.18 Assessment of catalase activity in cell lysates

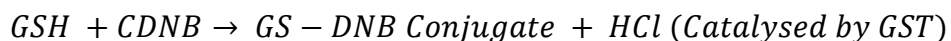
Catalase activity was determined in the same lysate samples using the method described by Maehly and Chance (1954). The assay quantifies the decomposition of hydrogen peroxide catalyzed by catalase.

5.4.19 Assessment of GST activity in cell lysates

GST is an important phase II detoxification enzyme that protects cells against oxidative stress by catalyzing the conjugation of reduced glutathione to electrophilic substrates, thereby facilitating their elimination. Under hyperglycemic conditions, excessive ROS may impair GST activity. Therefore, measurement of glutathione S-transferase activity serves as an indicator of cellular antioxidant status and therapeutic efficacy.

Principle

The glutathione S-transferase activity assay is based on the enzyme-catalyzed conjugation of reduced glutathione with an electrophilic substrate, 1-chloro-2,4-dinitrobenzene. In the presence of GST, reduced glutathione reacts with 1-chloro-2,4-dinitrobenzene (CDNB) to form S-(2,4-dinitrophenyl) glutathione, resulting in an increase in absorbance at 340 nm.



The rate of increase in absorbance is directly proportional to enzyme activity.

Procedure

GST activity was determined using the method described by Kushwaha et al. (2024). The reaction mixture contained 2 mL of 0.3 M potassium phosphate buffer (pH 6.35), 75 μ L of 30 mM CDNB, 725 μ L of distilled water, and 0.1 mL of cell homogenate. The mixture was vortexed and pre-incubated at 37 °C for 10 minutes. The reaction was initiated by adding 100 μ L of 30 mM reduced glutathione solution.

Absorbance was recorded at 340 nm at 30-second intervals for 2 minutes using a UV-visible spectrophotometer (Shimadzu UV-1800, Shimadzu, Kyoto, Japan). Enzyme activity was calculated using a molar extinction coefficient of $9.6 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ and expressed as units per milligram of protein. One unit of GST activity was defined as the amount of enzyme required to catalyze the conjugation of 1 nmol of GS-CDNB per minute.

5.5 *In vivo* evaluation of AEGN-3

5.5.1 Procurement and Acclimatization of experimental animals

For the *in vivo* assessment of AEGN-3, 46 adult female Sprague Dawley rat (6-8 weeks of age; body weight 180-200 g) were procured in 2024 from the National Institute of Pharmaceutical Education and Research, Mohali, India. Upon arrival at the animal facility of Lovely Professional University, the animals were housed in polypropylene cages (6-8 animals per cage) under standardized environmental conditions, including controlled temperature (22-25 °C), relative humidity (50-55%), and a 12-hour light/12-hour dark cycle. Animals were provided ad libitum access to a standard laboratory pellet diet and filtered drinking water.

Prior to initiation of the experimental protocol, all animals underwent a 7-day acclimatization period to adapt to laboratory conditions and minimize stress-related physiological variations. All experimental procedures were conducted in accordance with the guidelines of the

Committee for the Control and Supervision of Experiments on Animals. Ethical approval for the study was obtained from the Institutional Animal Ethics Committee (Approval No. LPU/IAEC/2024/70) of Lovely Professional University, Punjab, India.

5.5.2 Animal grouping and induction of diabetes

Following the acclimatization period, animals were randomly allocated into two dietary regimens. The control group received a normal pellet diet (NPD) and served as the normoglycemic control, whereas the experimental group was administered HFD for 14 days to induce insulin resistance.

The composition of the HFD was prepared according to the method described by Gaikwad et al. (2010). The formulation consisted of powdered normal pellet diet (365 g/kg), lard (310 g/kg), casein (250 g/kg), cholesterol (10 g/kg), vitamin and mineral mixture (60 g/kg), DL-methionine (3 g/kg), yeast powder (1 g/kg), and sodium chloride (1 g/kg). This formulation provided a macronutrient distribution of approximately 58% fat, 25% protein, and 17% carbohydrate, consistent with previously established protocols for diet-induced insulin resistance (Srinivasan et al., 2005).

After one week of HFD feeding (day 1-7), diabetes was induced in the HFD-fed group by a single intraperitoneal injection of freshly prepared streptozotocin at a dose of 35 mg/kg body weight. STZ was dissolved in 0.1 M citrate buffer (pH 4.4) immediately prior to administration to maintain stability. Following streptozotocin injection on day 8, the HFD regimen was continued for an additional week (day 8-14). At the end of the 14-day induction period, fasting blood glucose levels were measured using a glucometer. Rats exhibiting fasting blood glucose levels greater than 250 mg/dL were considered diabetic and included in subsequent experimental evaluations (Gaikwad et al., 2010; Rehman et al., 2023).

Experimental design:

To examine the effect of AEGN-3, we designed a total of 6 experimental groups.

- **Group 1:** Normal control rats consisted of non-diabetic, non-fatty rats receiving 0.5% normal saline
- **Group 2:** Diabetic control rats fed a high-fat diet (HFD) and injected with a single dose of STZ (35 mg/kg, i.p.)
- **Group 3:** Diabetic control rats that received acarbose at 50 mg/kg (p.o.)
- **Group 4:** Diabetic control rats that received guar gum at 200 mg/kg (p.o.)

- **Group 5:** Diabetic control rats that received AEGN-3 at 25 mg/kg (p.o.)
- **Group 6:** Diabetic control rats that received AEGN-3 at 50 mg/kg (p.o.)

Table 5.1: Experimental grouping and treatment regimen for evaluation of the antidiabetic potential of AEGN-3 in HFD-fed, STZ-induced diabetic rats.

S. No	Group Name	Diet + Dose and route of drug treatment	No. of animals
Group 1	Non-diabetic control (Vehicle)	NPD for 5 weeks + 0.5% Normal saline	6
Group 2	Negative control (diabetic group)	HFD for 5 weeks + 35 mg/kg STZ (i.p.)	8
Group 3	Diabetic + Acarbose	HFD for 5 weeks + 35 mg/kg STZ + 50 mg/kg of acarbose (p.o.)	8
Group 4	Diabetic + Guar gum	HFD for 5 weeks + 35 mg/kg STZ + 200 mg/kg of guar gum (p.o.)	8
Group 5	Diabetic + AEGN-3 (Low-dose)	HFD for 5 weeks + 35 mg/kg STZ + 25 mg/kg of AEGN-3 (p.o.)	8
Group 6	Diabetic + AEGN-3 (High-dose)	HFD for 5 weeks + 35 mg/kg STZ + 50 mg/kg of AEGN-3 (p.o.)	8

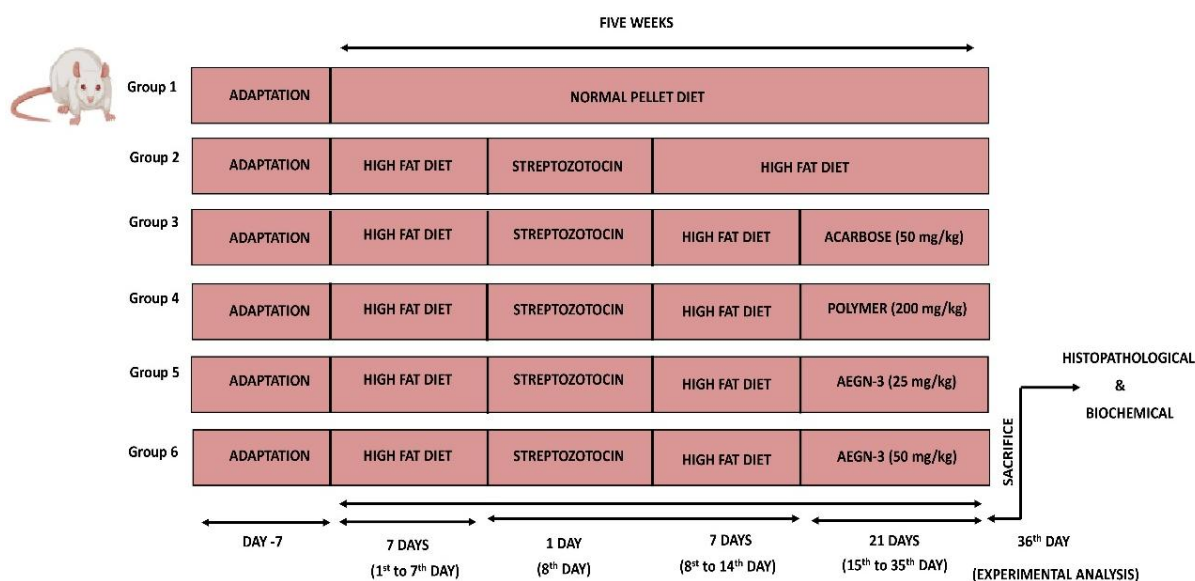


Figure 5.1: Schematic representation of the experimental design for evaluating the therapeutic effects of AEGN-3 in an HFD-fed, STZ-induced diabetic rat model.

5.5.3 Assessment of body weight in diabetic rats

Body weight of all experimental animals was recorded at weekly intervals throughout the study duration, from baseline (day 1) until termination of the experiment (day 35). Measurements were performed using a calibrated electronic balance to ensure accuracy and reproducibility. Monitoring of body weight served as an indicator of metabolic status and overall health, particularly in HFD-fed, STZ-induced diabetic rats, where weight loss is commonly associated with impaired glucose utilization and metabolic dysregulation (Srinivasan et al., 2005).

5.5.4 Assessment of blood glucose levels in diabetic rats

Fasting blood glucose levels were measured at predetermined time points during the study. On day 1, prior to initiation of dietary intervention, baseline glucose levels were recorded to confirm normoglycemic status in all animals. Following HFD feeding and STZ administration, blood glucose levels were reassessed on day 14 to confirm successful induction of diabetes. Animals exhibiting fasting blood glucose levels greater than 250 mg/dL were considered diabetic and included in subsequent treatment protocols.

Treatment with AEGN-3 was initiated on day 15 and continued until day 35. The antihyperglycemic efficacy of AEGN-3 was evaluated by measuring fasting blood glucose levels on day 35. Approximately 0.1 mL of blood was collected from the tail vein using a sterile

lancet, and glucose concentrations were determined using a CareSens II Plus glucometer (i-SENS Inc., Seoul, Korea) (Srinivasan et al., 2005).

5.5.5 Estimation of diarrhoea frequency

Diarrhoea incidence and severity were monitored daily from day 15 to day 35 in all experimental groups. Animals were housed in cages fitted with a wire mesh grid, allowing fecal matter to pass through onto a collection plate placed beneath the cage. The number of intact fecal pellets and the presence of watery halos indicative of diarrhoeal discharge were recorded daily. Diarrhoea severity was quantified using a modified scoring system based on stool consistency and moisture content, as described by Kumar et al. (2012). Fecal samples were visually examined and assigned scores to determine the extent of diarrhoeal response. The detailed scoring criteria are presented in Table 5.2.

Table 5.2: Scoring criteria for the assessment of diarrhoea severity in experimental animals. Diarrhoea was evaluated based on stool consistency and moisture content. Fecal samples were observed and assigned scores to quantify the severity of diarrhoeal response.

Score	Description
0	Normal, well-formed feces or no fecal output
1	Well-formed feces with slight moisture
2	Soft, shapeless feces indicating mild to moderate diarrhoea
3	Unshaped feces with high liquid content, indicative of severe diarrhoea

5.5.6 Assessment of animal survival during 35-day experimental period

Animal survival was monitored daily throughout the 35-day experimental duration to evaluate the tolerability of diabetes induction and treatment interventions. Mortality was recorded using a binary scoring system (0 indicating no mortality; 1 indicating mortality). Animals were observed routinely for clinical signs of distress, including reduced mobility, piloerection, altered feeding behavior, or abnormal posture.

5.5.7 Assessment of hepatotoxicity markers in diabetic rats

The liver is central to glucose homeostasis, detoxification, and intermediary metabolism. Chronic hyperglycemia and associated oxidative stress in diabetes mellitus can impair hepatic function, resulting in altered serum liver enzyme profiles. Therefore, serum hepatic biomarkers were evaluated to determine the effect of AEGN-3 on liver function.

The following biochemical parameters were assessed: total bilirubin, Serum Glutamic-Oxaloacetic Transaminase (SGOT), Serum Glutamic-Pyruvic Transaminase (SGPT), Alkaline Phosphatase (ALP), Gamma-Glutamyl Transferase (GGT), and LDH. Alterations in these markers may indicate hepatocellular injury, cholestasis, or metabolic dysfunction commonly associated with diabetic conditions.

Procedure

Following overnight fasting, animals were anesthetized with thiopentone sodium (50 mg/kg, intraperitoneal) to induce light surgical anesthesia. Adequate anesthesia was confirmed by the absence of the paw withdrawal reflex. Blood samples were collected from the retro-orbital plexus under aseptic conditions.

Samples were centrifuged at $3000 \times g$ for 10 minutes to obtain serum (Rodríguez et al., 2018). Serum biochemical parameters were quantified using commercially available spectrophotometric assay kits (Randox Laboratories, United Kingdom) according to the manufacturer's instructions.

5.5.8 Assessment of nephrotoxicity markers in diabetic rats

Persistent hyperglycemia contributes to progressive renal dysfunction, commonly referred to as diabetic nephropathy. Renal function was assessed by measuring serum creatinine, uric acid, and urea levels. Elevated creatinine indicates impaired glomerular filtration rate, whereas increased urea and uric acid levels reflect compromised renal excretory function.

Procedure

Serum samples obtained as described above were analyzed for nephrotoxicity markers using commercially available spectrophotometric assay kits (Randox Laboratories, United Kingdom) following standardized protocols (Matboli et al., 2017).

5.5.9 Assessment of lipid profile and toxicity markers in diabetic rats

Diabetes mellitus is frequently associated with dyslipidemia characterized by elevated total cholesterol, triglycerides, very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) levels, along with reduced high-density lipoprotein (HDL) concentrations. Such lipid abnormalities contribute to atherogenesis and cardiovascular complications. Serum lipid parameters, including total cholesterol, triglycerides, VLDL, LDL, and HDL, were evaluated to assess metabolic alterations and the therapeutic efficacy of AEGN-3.

Procedure

Lipid profile parameters were determined using commercially available spectrophotometric assay kits (Randox Laboratories, United Kingdom) according to the manufacturer's guidelines, as previously described (Firdous et al., 2021).

5.5.10 Tissue isolation, organ weight, and histopathological assessment of liver, kidney, pancreas, and heart

At the end of the experimental period, all animals were anesthetized, and the liver, kidney, pancreas, and heart tissues were carefully excised from each experimental group. The tissues were trimmed to remove adherent fat and connective tissue, rinsed with normal saline, and blotted dry. Immediately after excision, organ weights were recorded using a calibrated digital balance (Shimadzu AX200, Kyoto, Japan).

Following isolation and weighing, liver and kidney tissues were fixed in 10% neutral buffered formalin for 24-48 hours to preserve tissue architecture. The tissues were then processed through graded alcohol dehydration, cleared in xylene, and embedded in paraffin wax. Sections of 5 μm thickness were cut using a Leica RM2235 manual rotary microtome (Leica Biosystems, Germany) and mounted on poly-L-lysine-coated glass slides. The sections were stained with hematoxylin and eosin (H&E) for histopathological examination. Liver sections were evaluated for inflammation, hepatocyte damage, and sinusoidal dilation, whereas kidney sections were examined for glomerular and tubular alterations, including glomerular space dilation and interstitial changes indicative of diabetic nephropathy.

Similarly, pancreatic and heart tissues were fixed in 10% neutral buffered formalin for 24-48 hours, followed by dehydration, clearing, and paraffin embedding. Thin sections (5 μm) were prepared using the same microtome and mounted on poly-L-lysine-coated slides. After H&E staining, pancreatic sections were assessed for islet morphology, acinar cell integrity, β -cell

destruction, vacuolization, and inflammatory infiltration. Heart sections were examined for myocardial fiber arrangement, cellular infiltration, vacuolization, structural disorganization, and degenerative or necrotic changes associated with diabetes-induced cardiotoxicity. All histopathological evaluations were performed under light microscopy by an experienced histopathologist blinded to the experimental groups to ensure unbiased analysis.

5.5.11 Tissue homogenization of liver, kidney, pancreas, and heart in diabetic rats

At the end of the experimental period, liver, kidney, pancreas, and heart tissues were excised from control and diabetic-treated animals under aseptic conditions. The tissues were immediately rinsed with ice-cold phosphate-buffered saline (PBS) to remove residual blood and debris, blotted dry, and weighed to determine wet tissue mass.

A 10% (w/v) tissue homogenate was prepared by homogenizing 1 g of tissue in 9 mL of ice-cold homogenization buffer containing 0.1 M Tris-HCl and 0.1 M EDTA (pH 7.4). β -mercaptoethanol was freshly added to the buffer immediately before use to prevent oxidative protein degradation and preserve enzyme activity. Homogenization was performed using a glass-Teflon homogenizer or a motorized tissue homogenizer. The homogenization vessel was maintained in an ice bath throughout the procedure to minimize thermal denaturation and proteolytic degradation. The homogenate was subsequently filtered through sterile cotton gauze to remove connective tissue fragments and fibrous debris.

The filtrate was centrifuged at 10,000 rpm for 5 minutes at 4 °C using a refrigerated centrifuge (REMI C-24) to separate the nuclear pellet from the supernatant. The post-nuclear supernatant was carefully collected and subjected to further centrifugation at $10,000 \times g$ for 10 minutes at 4 °C to isolate the mitochondrial pellet. The resulting post-mitochondrial supernatant, enriched in cytosolic and microsomal fractions, was collected and stored in labeled microcentrifuge tubes at -18 °C until subsequent biochemical analysis (Kushwaha et al., 2024).

5.5.12 Assessment of protein content using Lowry's method

Total protein concentration in tissue homogenates obtained from liver, kidney, pancreas, and heart was determined using the method described by Lowry et al. (1951). The previously established protocol was followed for all experimental groups. Protein concentration was calculated from a standard calibration curve and subsequently used to normalize enzymatic activities, which were expressed per milligram of protein.

5.5.13 Assessment of oxidative biomarkers in tissues

Oxidative stress markers were evaluated in tissue homogenates from all experimental groups. Lipid peroxidative damage was assessed by measuring MDA levels according to the method of Iqbal et al. (1996). SOD and catalase activities were determined using the methods described by Beyer et al. (1987) and Maehly and Chance (1954), respectively. GST activity was measured following the protocol described by Kushwaha et al. (2024). Enzyme activities were expressed relative to protein content.

5.5.14 Assessment of AChE and Xanthine oxidase activities in tissue homogenates

The activities of AChE and Xanthine oxidase were quantified in tissue homogenates to evaluate the modulatory effect of AEGN-3 on cholinergic and oxidative pathways. Enzymatic activities were determined according to standardized methods described by Rahimi et al. (2022). Results were expressed as units per milligram of protein.

5.5.15 Assessment of inflammatory mediators in tissue homogenates

Chronic hyperglycemia is associated with enhanced oxidative stress and activation of inflammatory signaling cascades, contributing to the progression of diabetic complications in multiple organs, including the liver, kidney, pancreas, and heart. Pro-inflammatory cytokines such as TNF- α and IL-6 promote insulin resistance, cellular injury, and tissue remodeling, whereas IL-10 exerts anti-inflammatory effects by suppressing cytokine-mediated inflammatory responses. Quantification of these mediators provides insight into the inflammatory status of diabetic tissues and the therapeutic efficacy of AEGN-3 (Alhazzani et al., 2025).

Procedure

To assess inflammatory mediators, the supernatants and tissue homogenates were used to quantify the pro-inflammatory cytokines TNF- α and IL-6, as well as the anti-inflammatory cytokine IL-10. These cytokine levels were measured using commercially available ELISA kits (R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. The assay was conducted in duplicate, and absorbance was read at the specified wavelength using a microplate reader.

Tissue homogenates and corresponding supernatants were used for the quantification of TNF- α , IL-6, and IL-10 levels. Cytokine concentrations were determined using commercially

available ELISA assay kits from R&D Systems (Minneapolis, Minnesota, United States) according to the manufacturer's instructions. All samples were analyzed in duplicate, and absorbance was measured at the specified wavelength using a microplate reader (MPM 6, Bio-Rad). Cytokine concentrations were calculated from standard calibration curves generated for each assay.

5.6. Network Pharmacology study

5.6.1 Retrieval of data from different databases for identifying potential targets

In this study, we utilized several tools for data mining to identify potential targets and genes of acarbose related to type 2 diabetes. These datasets were curated to remove redundant entries, focusing on common genes present in both conditions. The identified potential anti-diabetic genes underwent further analysis through protein-protein interaction (PPI) network construction, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, and Gene Ontology (GO) annotation followed by Database for Annotation, Visualization, and Integrated Discovery (DAVID) database to elucidate their biological significance and involvement in disease mechanisms. Subsequently, molecular docking was performed on the top 10 selected hub genes to evaluate their binding energy scores with acarbose. The four hub genes with the highest binding energy scores were then selected for molecular simulation analysis to assess the stability and dynamics of the acarbose-target complexes.

5.6.2 Identification of acarbose-associated targets from different databases

The potential targets were retrieved from several databases, including SwissTarget Prediction (<http://www.swisstargetprediction.ch/>), STITCH (<http://stitch.embl.de/>), BindingDB (<https://www.bindingdb.org/bind/index.jsp>), PharmMapper (<http://lilab.ecust.edu.cn/pharmmapper/>), and CTD (<https://ctdbase.org/>), accessed on May 27, 2024. The predicted targets were identified using the SMILES format of acarbose in BindingDB and SwissTarget Prediction, based on structural similarity principles. To identify the potential targets of acarbose, we initiated a search using the term "Acarbose" in the STITCH database. For additional target identification, we used the PharmMapper server by first retrieving the acarbose structure from PubChem, which was then uploaded to PharmMapper for pharmacophore mapping to obtain potential targets.

5.6.3 Identification of targets associated with type 2 diabetes from different databases

The search for potential targets in various databases, including Online Mendelian Inheritance in Man (OMIM), DISGENET, GeneCards, and MalaCards, was conducted using the key term 'type 2 diabetes' and was accessed on May 27, 2024. The lists generated after searching this key term in these databases were further normalized using UniProt (<http://www.uniprot.org/>).

5.6.4 Construction and analysis of PPI networks

Since proteins are crucial in determining how biological processes function, regulating molecular and cellular activities, and predicting the health or disease state of an organism, we aimed to analyse the interaction of target genes through PPIs. We selected only those PPI pathways linked to diabetes and its associated complications that could potentially support drug design and the artificial design of proteins. As previously discussed, the key search terms were 'Acarbose' and 'type 2 diabetes'. The entries obtained from UniProt were normalized and then processed using the GeneMANIA database (<https://genemania.org/>) to analyse closely related genes, focusing specifically on Homo sapiens. PPI network was constructed using Cytoscape (version 3.6.1), which facilitates the integration and visualization of data (Otasek et al., 2019).

5.6.5 Identification of hub genes using Cytoscape network analysis

To screen the top 10 hub genes from PPI network, we analysed network topological properties such as degree, betweenness centrality, and closeness centrality using the CytoHubba plugin within Cytoscape (Chin et al., 2014). This network analysis tool helped us identify hub genes from the PPI network for further investigation.

5.6.6 Gene Enrichment analysis

To elucidate the biological functions of target proteins and pathways related to type 2 diabetes, we conducted GO and KEGG enrichment analyses using the DAVID tools version 6.8 (Sherman et al., 2022). The KEGG pathway enrichment analysis revealed significantly enriched metabolic pathways associated with the targets when compared to the entire genome background. Only pathways with direct relevance to type 2 diabetes were retained for downstream interpretation. The statistical significance of enrichment was evaluated using a false discovery rate (FDR) control method, with Q-values computed at a threshold of $p < 0.05$ to minimize type I errors. All enrichment results were visualized using the Weishengxin Bioinformatics online platform (<https://www.bioinformatics.com.cn>), which enabled the graphical presentation of GO categories and KEGG pathways (Tang et al., 2023).

5.6.7 Molecular docking of Hub genes

Molecular docking was performed using AutoDock Vina to assess the interaction between acarbose targets and the core compound (Trott & Olson, 2010), using 3D crystal structures from the Protein Data Bank (accessed on 12 September 2024). The targets were prepared by removing water molecules, heteroatoms and default ligands. The polar hydrogen atoms and kollman charges were added by AutoDock Tools 1.5.7 to the core targets and then were converted into PDBQT for further docking (Park et al., 2006). The acarbose 3D molecular structure was retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). The downloaded structures were then converted into pdbqt format using a toolbox OPEN BABEL (O'Boyle et al., 2011). The docking procedure was repeated three times and the docking exhaustiveness was set at 20 to guarantee Vina produced the best possible results in an acceptable time span. Docking results were analysed by PyMol (Version 2.5.2), and the interactions were further visualized using Discovery Studio Visualizer (Discovery Studio Visualizer v.21.1.0.20298) (Baroroh et al., 2023).

5.6.8 Molecular simulation of selected Hub proteins

Using the GROMACS 2024.2 program, all protein targets from molecular docking studies were chosen for MD simulations (Lemkul, 2019), SwissParam server (Bugnon et al., 2023) was used to construct the topology of proteins and ligands, and CHARMM27 was chosen as an all-atom force field (Bjellmar et al., 2010). A conventional [number of particles (N), system volume (V), and temperature (T), NVT] and isobaric-isothermic ensemble were used to equilibrate the complex after it was solved using the TIP3P water model and neutralised with Na⁺ and Cl⁻ ions for 100 picoseconds (ps). At 300 K and 1 bar of pressure, the NPT group conducted the last MD run of protein-ligand complexes for 100 nanoseconds (ns). RMSD, root mean square fluctuations (RMSF) for the protein and ligand, and the radius of gyration (RoG) of the ligand in the complex were computed using the extracted data in order to comprehend the appropriate dynamic alterations of the protein-ligand complex at the molecular level.

5.6.9 Gene expression validation

To evaluate the tissue-specific expression patterns of the identified hub genes, we conducted RNA-seq expression analysis using the EasyGEO module of the eVITTA platform (<https://www.ncbi.nlm.nih.gov/geo/>) (Barrett et al., 2013). Relevant datasets were identified from the Gene Expression Omnibus (GEO) database using keywords such as *"liver," "pancreas," "adipose tissue," "skeletal muscle," "peripheral blood,"* and *"RNA-sequence*

expression." Datasets were included based on predefined criteria: availability of both healthy and disease samples, relevance to type 2 diabetes or metabolic conditions, and adequate sample size. The selected datasets for analysis were GSE15653, GSE20966, GSE29226, GSE55650, and GSE15932. Data from these datasets were manually input into the EasyGEO tool and analysed using the Limma statistical method. No fold-change threshold was applied, allowing the identification of all significantly differentially expressed genes. This approach facilitated a comprehensive assessment of hub gene expression across key tissues involved in type 2 diabetes pathophysiology.

Chapter 6

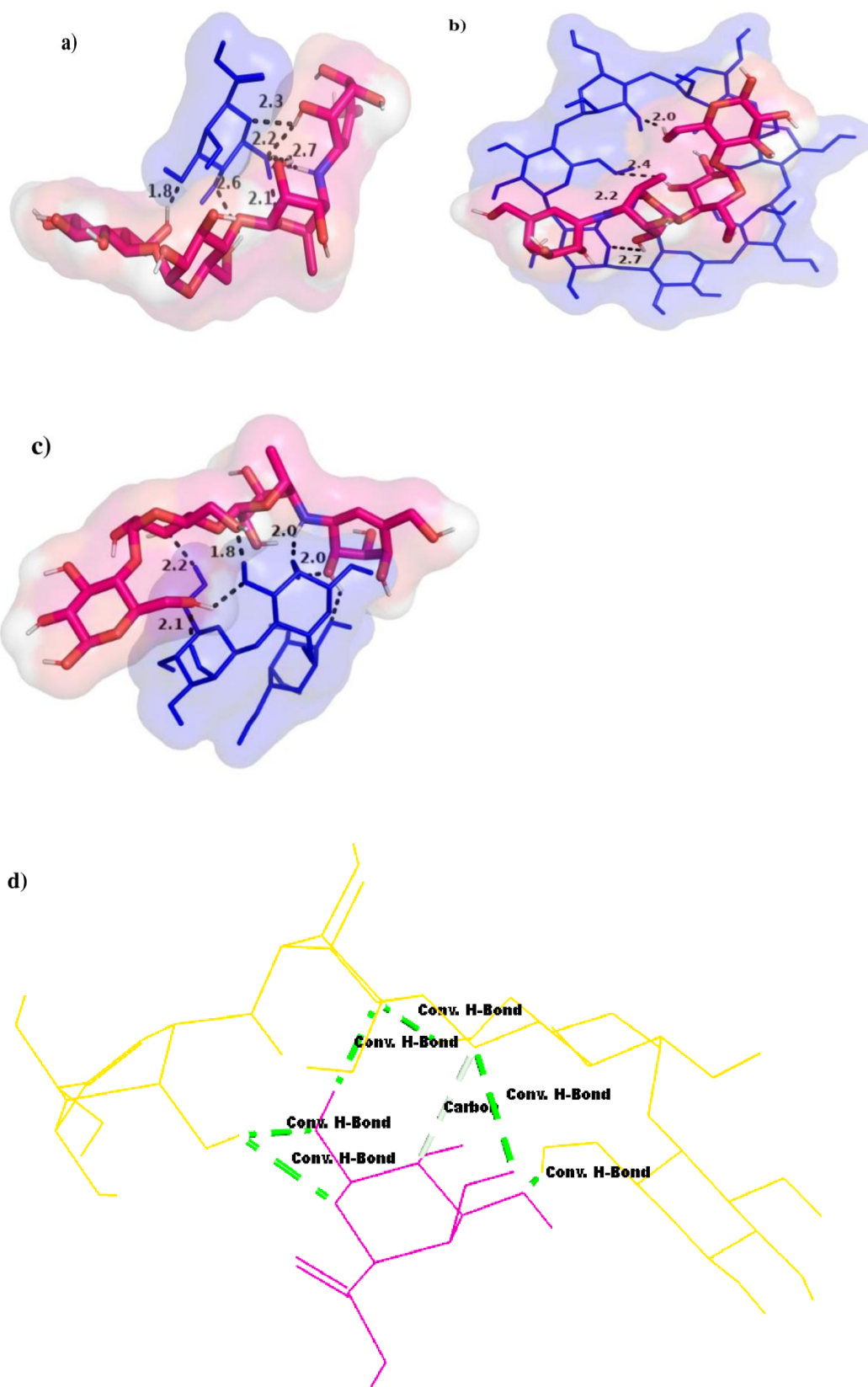
Result

6.1. Preliminary study

6.1.1 Molecular docking of acarbose with polymers

The binding potential, key interactions, and likely structures of polymers with acarbose were investigated by molecular docking studies employing AutoDock Tools (Figure 6.1a-f). The polymers were categorized based on their docking scores, as acarbose successfully formed complexes with selected polymers. Binding energies were interpreted with reference to common thresholds for small molecule-polymer interactions, where values around -5 kcal/mol indicate moderate binding, sufficient to stabilize the complex without overly restricting drug release, whereas values above -3 kcal/mol suggest weaker interactions that may allow faster release.

In brief, guar gum exhibited a binding energy of -3.9 kcal/mol, β -CD showed the maximum binding energy of -5.1 kcal/mol; and, pectin exhibited the lowest binding energy (Table 6.1). These findings indicate that β -CD forms the most stable complex with acarbose among the evaluated polymers, whereas guar gum demonstrates moderate interaction strength. Depending on the intended formulation objective, stronger binding by β -CD may enhance complex stability, while the moderate interaction exhibited by guar gum may be favorable for controlled or sustained release applications. Acarbose interacted favourably with the hydrophilic area of β -CD. Moreover, subsequent investigations revealed that β -CD and acarbose interact via conventional hydrogen bonds and two carbon-hydrogen bonds, whereas guar gum and acarbose form only conventional hydrogen bonds. The type and number of hydrogen bonds indicate a stable but not overly rigid interaction for guar gum, supporting its potential to form a nanoformulation that balances stability and drug release.



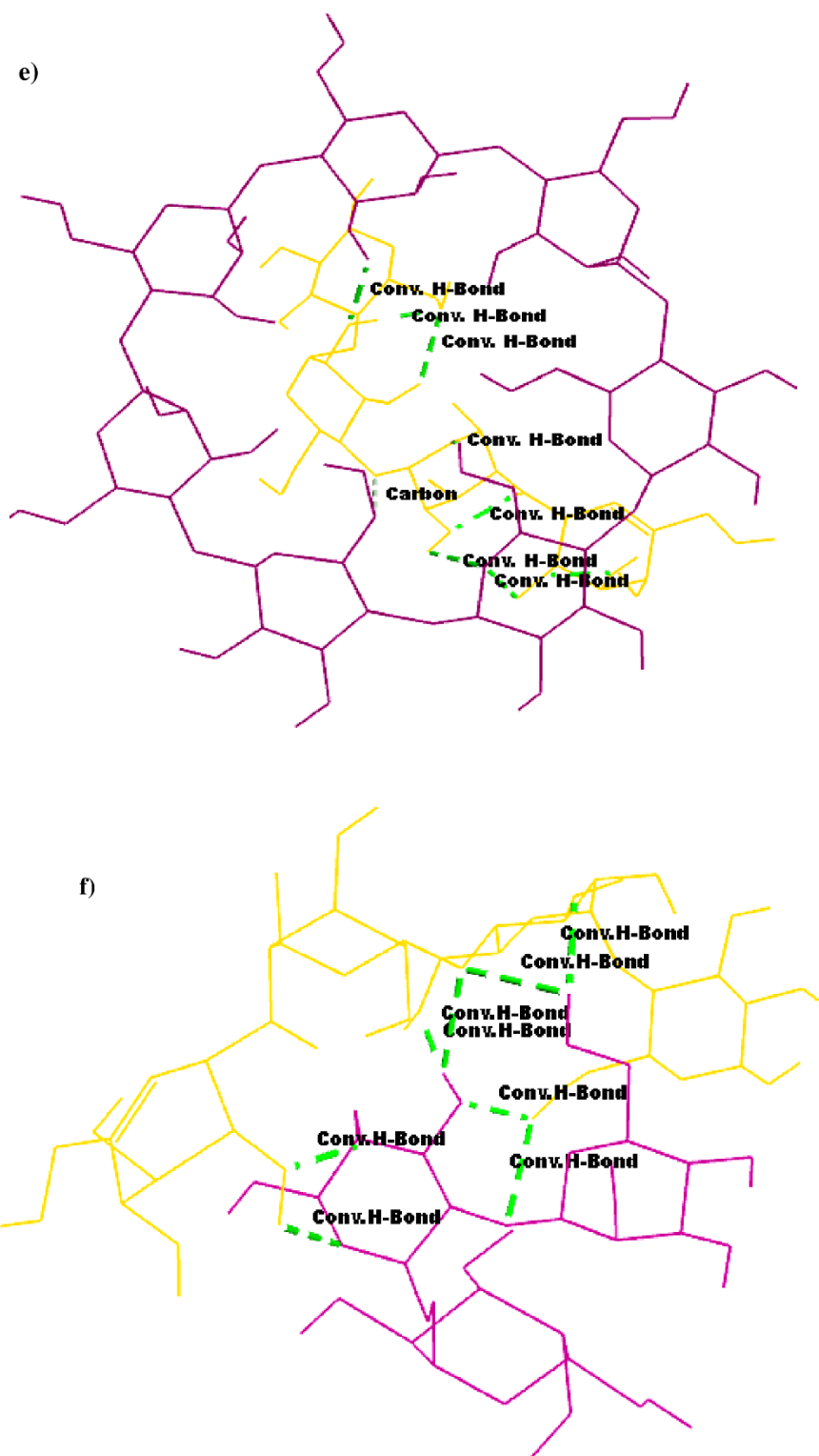


Figure 6.1: Molecular docking and interaction analysis of acarbose with (a) pectin, (b) β -CD, and (c) guar gum, and (d-f) corresponding 2D interaction profiles. Here, Conv. H-Bond: Conventional hydrogen-bond.

Table 6.1: Molecular docking scores of acarbose with selected polymers

Polymer	Docking score (kcal/mol)
Pectin	-2.8
β -CD	-5.1
Guar gum	-3.9

6.1.2 Molecular docking simulation

Using molecular dynamics modelling, the structural stability of acarbose and its complexes with various polymers was evaluated over 400 ns. To assess the relative stabilities of the complexes, RMSD values were calculated for each frame in the trajectory. All complexes maintained relatively constant RMSD values throughout the 400 ns period (Figure 6.2). The pectin-acarbose complex showed the least deviation, with an average RMSD of 0.96677 ± 0.38006 , followed by β -CD (1.07378 ± 0.50744) and guar gum (1.10171 ± 0.47759). These findings confirm that the predicted docking interactions are maintained over time, validating the molecular docking predictions. Notably, guar gum demonstrates sufficient stability for potential incorporation into a nanoformulation, without excessive rigidity that could hinder drug release.

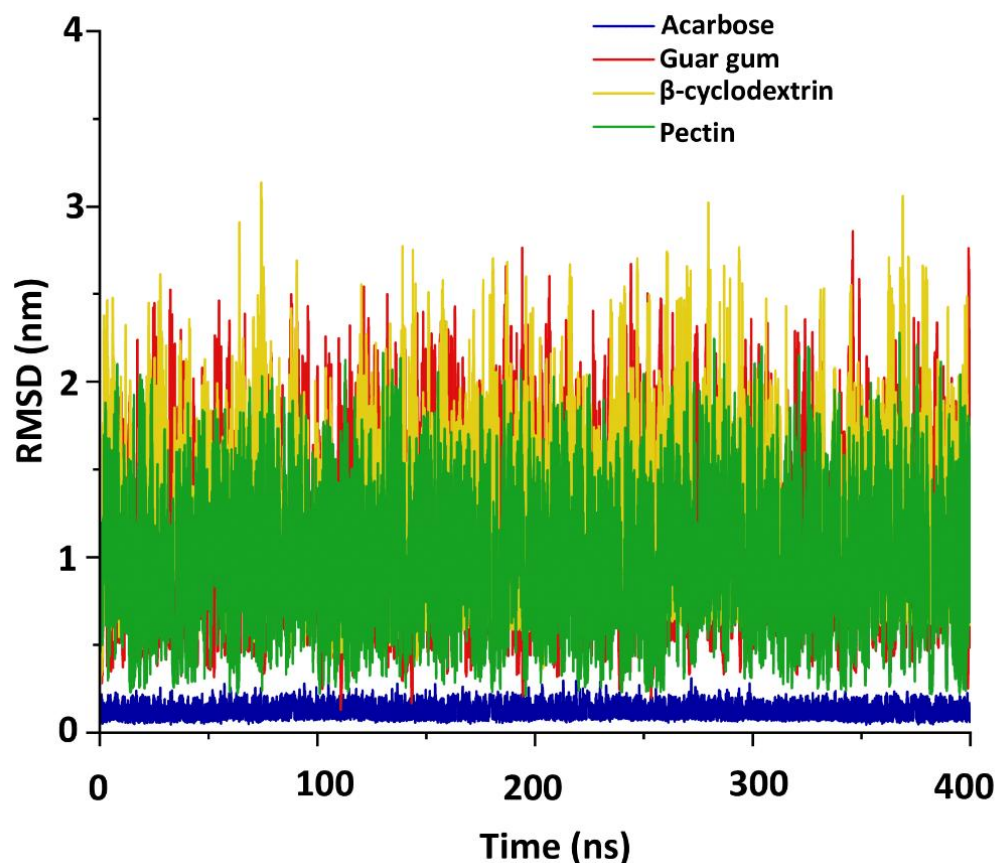


Figure 6.2: Molecular dynamics simulation of acarbose with selected polymers over a 400 ns time frame, showing RMSD profiles of the respective complexes.

6.1.3 Acarbose combination enhances α -glucosidase inhibitory activity

Molecular docking and simulation analyses demonstrated stable and biologically compatible polymer-acarbose interactions; therefore, the inhibitory potential of acarbose-polymer combinations against α -glucosidase was evaluated at varying ratios to assess their effects on enzymatic activity. Acarbose alone exhibited the highest inhibitory activity compared to all polymers, whether used individually or in combination (Figure 6.3). At 100% concentration, pectin, β -CD, and guar gum exhibited significantly lower inhibition than acarbose by 1.34-fold ($p < 0.01$), 2.28-fold ($p < 0.001$), and 1.28-fold ($p < 0.01$), respectively.

Combination ratios demonstrated ratio-dependent inhibition. The 25:75 drug:polymer ratio showed moderate inhibition, though still lower than acarbose by 1.48-fold ($p < 0.01$), 1.53-fold ($p < 0.01$), and 1.44-fold ($p < 0.01$) for pectin, β -CD, and guar gum, respectively. The 75:25 ratio exhibited comparatively higher inhibition among combinations, with reductions of 1.08-fold ($p < 0.05$), 1.16-fold ($p < 0.05$), and 1.04-fold ($p < 0.05$) relative to acarbose. At the 50:50

ratio, α -glucosidase inhibition was significantly increased compared to the 25:75 ratio, but remained lower than acarbose by 1.12-fold ($p < 0.05$), 1.29-fold ($p < 0.01$), and 1.20-fold ($p < 0.05$) for pectin, β -CD, and guar gum, respectively. Notably, combinations containing a higher proportion of acarbose (75:25) demonstrated inhibition levels closer to the pure drug, indicating that polymer incorporation did not abolish enzymatic activity.

Considering that docking analyses revealed moderate binding energies, the retained inhibitory activity across combinations supports the biological relevance of these interaction strengths, suggesting that complex formation does not excessively restrict drug availability at the enzyme interface. Overall, while all combinations showed lower inhibition than 100% acarbose, the 75:25 ratio emerged as the most effective among tested combinations. These findings provide experimental support for the in-silico predictions and justify the selection of this ratio for subsequent biological evaluations.

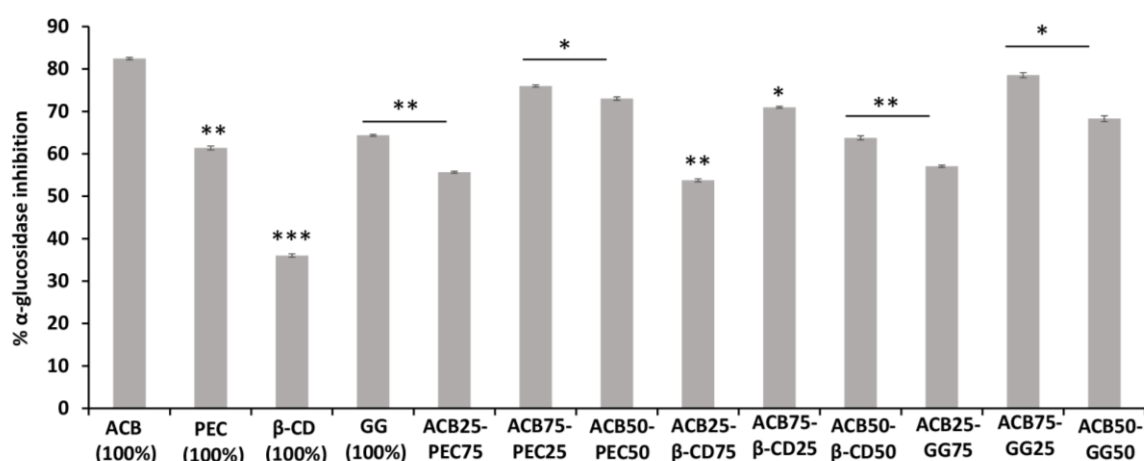


Figure 6.3: % inhibition of α -glucosidase by acarbose (ACB), polymers (PEC: pectin, β -CD: β -cyclodextrin, GG: guar gum), and their combinations at different ratios (100%, 25:75, 75:25, and 50:50 drug:polymer). Data are presented as Mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance compared to acarbose is indicated by * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

6.1.4 Acarbose combination enhances α -amylase inhibitory activity

Next, the inhibitory potential of acarbose in combination with different polymers on α -amylase activity was evaluated. Acarbose alone demonstrated the highest α -amylase inhibition

compared to pectin, β -CD, and guar gum (Figure 6.4). At 100% concentration, pectin, β -CD, and guar gum showed significantly lower inhibition than acarbose by 1.34-fold ($p < 0.01$), 2.45-fold ($p < 0.001$), and 1.11-fold ($p < 0.05$), respectively.

Combination ratios exhibited ratio-dependent effects on α -amylase inhibition. At the 25:75 drug:polymer ratio, inhibitory activity was enhanced compared to polymers alone, but remained lower than acarbose by 1.64-fold ($p < 0.01$), 2.71-fold ($p < 0.001$), and 1.55-fold ($p < 0.01$) for pectin, β -CD, and guar gum, respectively. At the 75:25 ratio, pectin and β -CD showed 1.05-fold ($p < 0.05$) and 1.08-fold ($p < 0.05$) lower inhibition than acarbose, while guar gum exhibited no significant difference from acarbose, indicating a more effective polymer-drug interaction at this ratio. Similarly, the 50:50 ratio displayed increased inhibition relative to the 25:75 ratio, but remained lower than acarbose by 1.30-fold ($p < 0.01$), 1.23-fold ($p < 0.01$), and 1.14-fold ($p < 0.05$) for pectin, β -CD, and guar gum, respectively.

Inhibition increased with higher acarbose proportion, with the 75:25 formulation exhibiting activity comparable to the pure drug, particularly in the guar gum combination where no significant difference was observed. The sustained α -amylase inhibition across combination groups indicates that polymer interaction does not substantially restrict drug accessibility at the enzyme active site. These findings are consistent with the α -glucosidase results and further support the functional compatibility of the selected drug-polymer ratios.

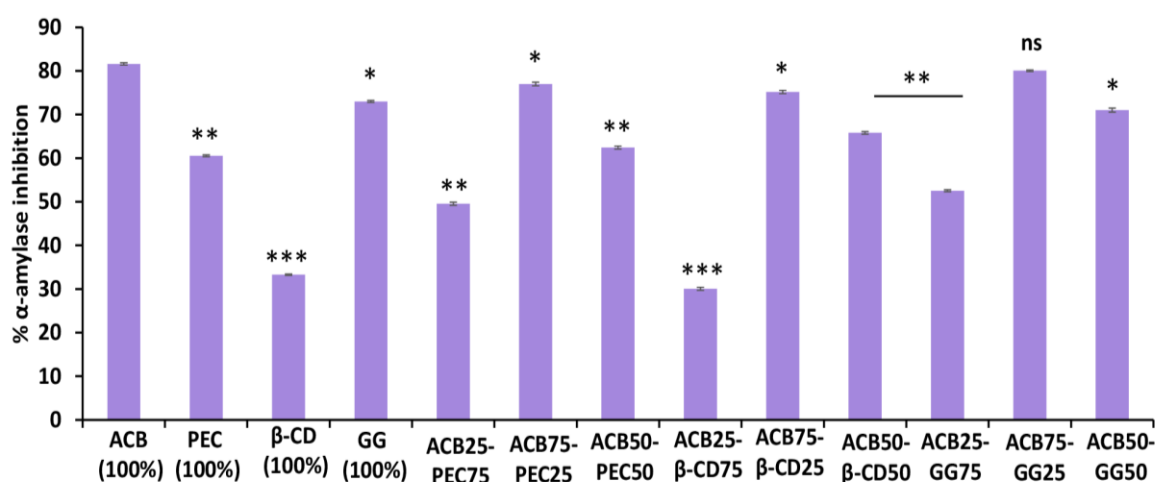


Figure 6.4: % inhibition of α -amylase by acarbose (ACB), polymers (PEC: pectin, β -CD: β -cyclodextrin, GG: guar gum), and their combinations at different ratios (100%, 25:75, 75:25, and 50:50 drug:polymer). Data are presented as Mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple

comparison post hoc test. Statistical significance compared to acarbose is indicated by * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. ns represents non-significant.

6.1.5 Acarbose combination enhances DPPH radical scavenging activity

The results shown in Figure 6.5 pertain to the effects of different combinations on DPPH radical scavenging activity. Pectin and guar gum at 100% concentration showed significant DPPH radical scavenging activity, increasing by 1.26-fold ($p < 0.01$) and 1.17-fold ($p < 0.01$), respectively, while β -CD reduced scavenging by 0.36-fold ($p < 0.001$) compared to acarbose alone.

Combinations of acarbose with pectin and guar gum at a 25:75 ratio showed enhanced scavenging by 1.39-fold ($p < 0.001$) and 1.32-fold ($p < 0.01$), respectively, while β -CD reduced activity ($p < 0.001$). At a 75:25 ratio, pectin increased scavenging by 1.19-fold ($p < 0.01$), whereas guar gum showed no significant change and β -CD reduced scavenging activity ($p < 0.001$). The 50:50 combination increased scavenging by 1.51-fold ($p < 0.001$) for pectin and 1.46-fold ($p < 0.001$) for guar gum, while the β -CD combination showed no significant difference compared to acarbose alone. Among the tested formulations, the 25:75 ratio demonstrated the highest DPPH scavenging activity for pectin and guar gum combinations, whereas β -CD consistently showed comparatively lower activity across ratios. Overall, DPPH scavenging activity was retained or enhanced in selected acarbose-polymer combinations, indicating that polymer incorporation does not adversely affect antioxidant performance. The preservation of radical scavenging capacity across combinations is consistent with the predicted non-restrictive polymer-drug interactions observed in the in-silico analyses.

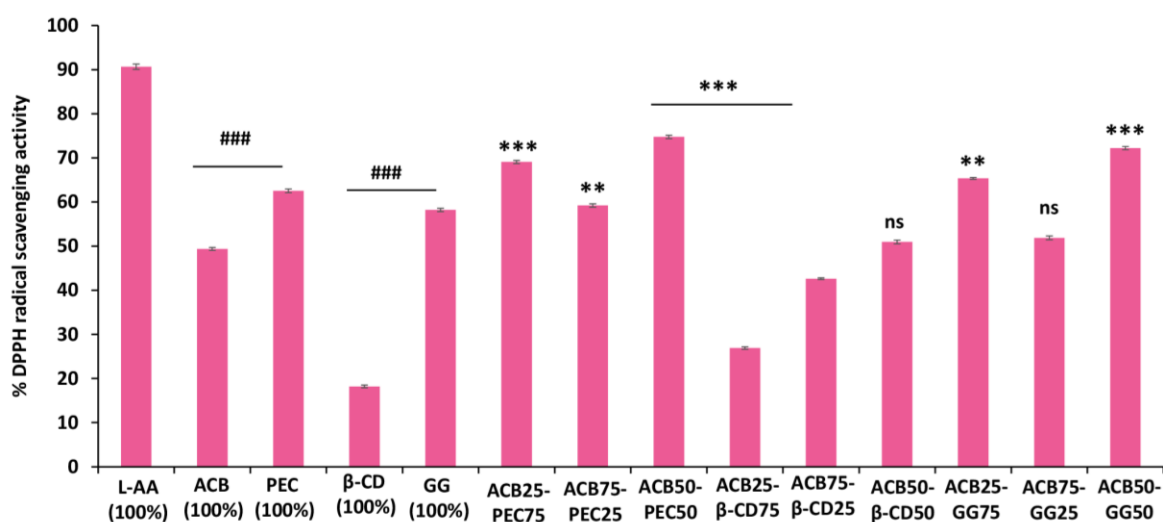


Figure 6.5: % inhibition of DPPH radicals by L-Ascorbic acid, acarbose (ACB), polymers (PEC: pectin, β -CD: β -cyclodextrin, GG: guar gum), and their combinations at different ratios (100%, 25:75, 75:25, and 50:50 drug:polymer). Data are presented as mean $\pm$ SEM (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated by #p < 0.05, ##p < 0.01, ###p < 0.001 compared to positive control, and *p < 0.05, **p < 0.01, ***p < 0.001 compared to acarbose. ns represent not significant.

6.1.6 Acarbose combination enhances ABTS radical scavenging activity

Next, we evaluated the potential of these combinations to scavenge ABTS radical cation. The data revealed that pectin and guar gum at 100% concentration increased ABTS radical scavenging by 1.43-fold (p < 0.001) and 1.24-fold (p < 0.01), respectively, while β -CD reduced scavenging by 3.10-fold (p < 0.001) compared to acarbose alone (Figure 6.6).

Combinations of acarbose with pectin and guar gum at a 25:75 ratio showed significant increases in ABTS scavenging by 1.55-fold (p < 0.001) and 1.41-fold (p < 0.001), while β -CD reduced scavenging by 1.89-fold (p < 0.001). At a 75:25 ratio, pectin and guar gum increased scavenging by 1.41-fold (p < 0.001) and 1.20-fold (p < 0.01), while β -CD showed a 1.47-fold reduction (p < 0.001). Similarly, at the 50:50 ratio, pectin and guar gum increased scavenging by 1.59-fold (p < 0.001) and 1.48-fold (p < 0.001), respectively, whereas β -CD reduced scavenging by 1.47-fold (p < 0.001). Among the tested formulations, the 25:75 ratio exhibited the highest ABTS scavenging activity for pectin and guar gum combinations, while β -CD consistently demonstrated comparatively lower activity across ratios. Overall, ABTS radical scavenging was retained or enhanced in selected acarbose-polymer combinations, indicating that polymer incorporation does not adversely affect antioxidant performance.

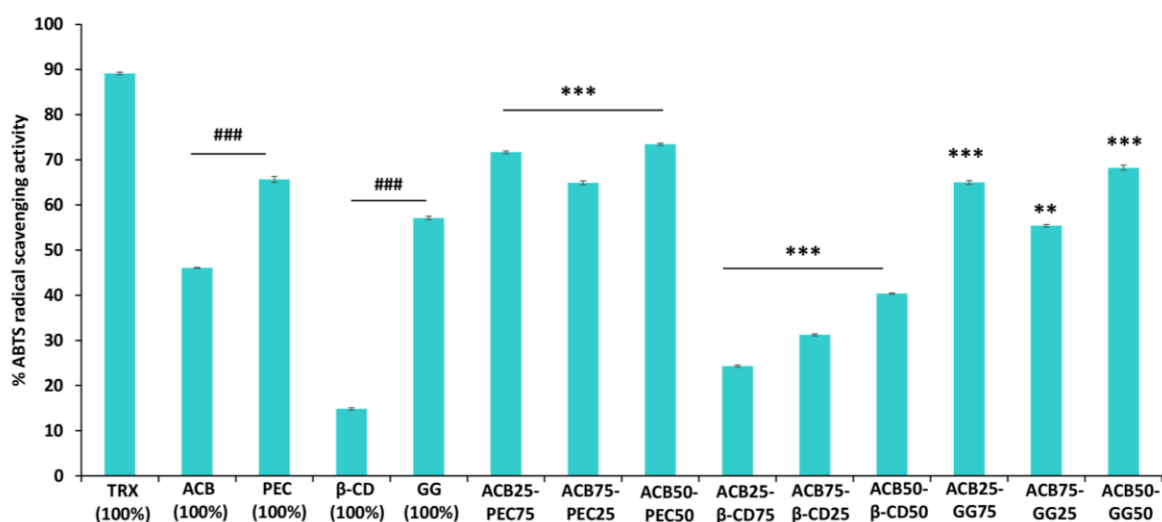


Figure 6.6: % inhibition of ABTS radicals by Trolox (TRX), acarbose (ACB), polymers (PEC: pectin, β-CD: β-cyclodextrin, GG: guar gum), and their combinations at different ratios (100%, 25:75, 75:25, and 50:50 drug:polymer). Data are presented as mean ± SEM (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated by #p < 0.05, ##p < 0.01, ###p < 0.001 compared to positive control, and *p < 0.05, **p < 0.01, ***p < 0.001 compared to acarbose.

6.1.7 Acarbose combination enhances Fe²⁺ chelation activity

As Fe²⁺ promotes ROS formation, its chelation is vital for reducing oxidative stress. All polymers, alone or in combination with acarbose, showed greater Fe²⁺ chelation than acarbose alone (Figure 6.7). Specifically, pectin, β-CD, and guar gum increased Fe²⁺ chelation by 2.41-fold (p < 0.001), 1.14-fold (p < 0.05), and 1.67-fold (p < 0.01), respectively.

When combined with acarbose at a 25:75 ratio, the chelation ability further increased by 2.16-fold (p < 0.001) for pectin, 2.00-fold (p < 0.001) for β-CD, and 2.11-fold (p < 0.001) for guar gum. At a 75:25 ratio, the enhancement was 1.92-fold (p < 0.001), 1.42-fold (p < 0.01), and 1.85-fold (p < 0.001), respectively, compared to 100% acarbose. The 50:50 ratio showed significant increases in chelation: 2.20-fold (p < 0.001) for pectin, 1.72-fold (p < 0.001) for β-CD, and 2.16-fold (p < 0.001) for guar gum. Among the tested formulations, the 25:75 and 50:50 ratios demonstrated comparatively higher chelation capacity than the 75:25 ratio. The consistent enhancement of Fe²⁺ chelation across acarbose-polymer combinations indicates that polymer interaction does not impair, and may contribute to metal-binding activity.

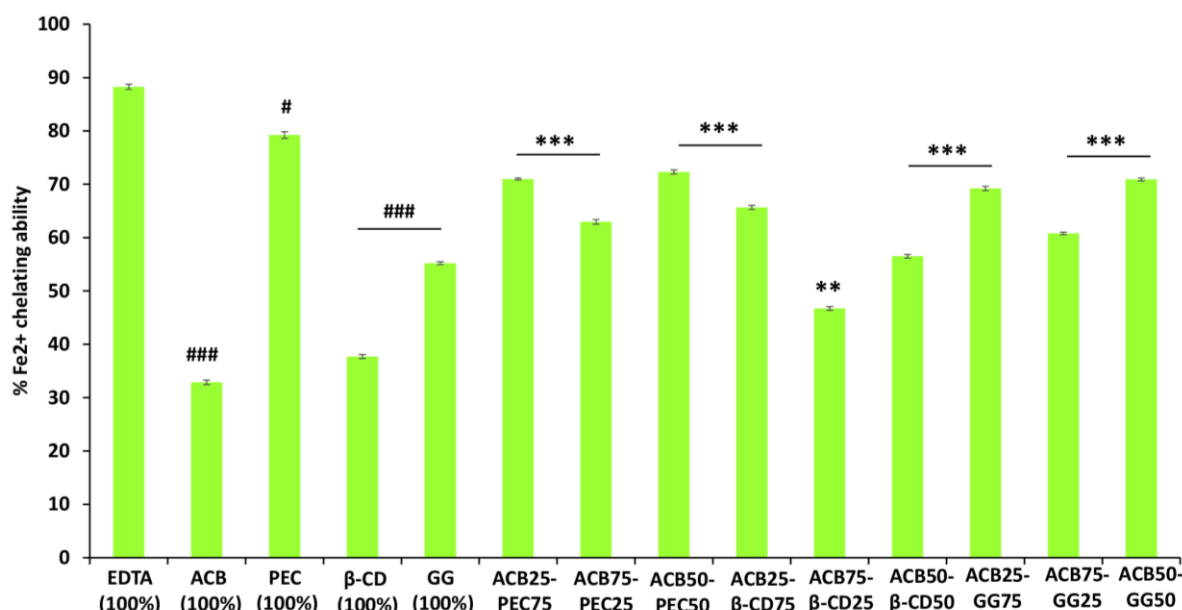


Figure 6.7: % Fe²⁺ chelation ability of Ethylenediaminetetraacetic acid (EDTA), acarbose (ACB), polymers (PEC: pectin, β-CD: β-cyclodextrin, GG: guar gum), and their combinations at different ratios (100%, 25:75, 75:25, and 50:50 drug:polymer). Data are presented as mean ± SEM (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated by #p < 0.05, ##p < 0.01, ###p < 0.001 compared to positive control, and *p < 0.05, **p < 0.01, ***p < 0.001 compared to acarbose.

6.1.8 Acarbose combination enhances nitric oxide radical scavenging activity

Subsequently, it was imperative to analyze the scavenging activity of nitric oxide radical as its reaction with superoxide leads to the formation of peroxynitrite, which further causes oxidative damage to lipids, proteins, and DNA. As shown in Figure 6.8, pectin and guar gum at 100% concentration increased nitric oxide radical scavenging by 1.16-fold (p < 0.01) and 1.10-fold (p < 0.05), respectively, while β-CD reduced scavenging (p < 0.05) compared to acarbose.

The combination of acarbose with pectin and guar gum enhanced scavenging by 1.18-fold (p < 0.01) and 1.09-fold (p < 0.05), respectively, while β-CD showed a reduction in scavenging (p < 0.05) at 25:75 ratio. Likewise, pectin and β-CD increased scavenging by 1.10-fold (p < 0.05) and 1.05-fold (p < 0.05), respectively, while the combination of acarbose with guar gum at 75:25 ratio significantly reduced scavenging activity (p < 0.05). The 50:50 combination of acarbose with pectin, β-CD, and guar gum showed significant increases in scavenging activity: 1.12-fold (p < 0.01) for pectin, 1.09-fold (p < 0.05) for β-CD, and 1.11-fold (p < 0.01) for guar

gum compared to 100% acarbose. Overall, pectin and guar gum, particularly at the 25:75 ratio, exhibited the highest nitric oxide scavenging activity, whereas β -CD showed comparatively lower effects. The 50:50 combination also maintained substantial activity, and these findings indicate that polymer incorporation does not impair the radical-scavenging ability of acarbose and aligns with the compatibility observed in the in-silico interaction analysis.

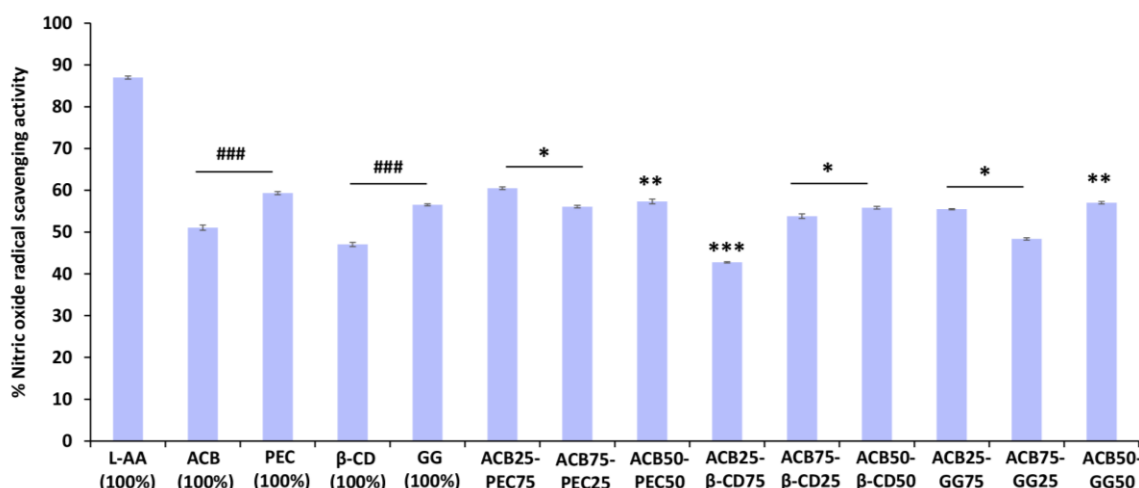


Figure 6.8: % inhibition of nitric oxide radicals by L-ascorbic acid (L-AA), acarbose (ACB), polymers (PEC: pectin, β -CD: β -cyclodextrin, GG: guar gum), and their combinations at different ratios (100%, 25:75, 75:25, and 50:50 drug:polymer). Data are presented as mean $\pm$ SEM (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated by #p < 0.05, ##p < 0.01, ###p < 0.001 compared to positive control, and *p < 0.05, **p < 0.01, ***p < 0.001 compared to acarbose.

6.1.9 Maintenance and experimental setup of *Drosophila melanogaster*

Following the non-enzymatic antioxidant assays, pectin and guar gum combinations demonstrated superior radical-scavenging activity compared to the β -CD combination. However, to comprehensively evaluate the *in vivo* relevance and comparative potential of all prepared combinations, each combination was further assessed in a H_2O_2 -induced oxidative stress model using *Drosophila melanogaster*. As depicted in Figure 6.9, *Drosophila melanogaster* was maintained in stock culture bottles containing standard cornmeal-agar medium. For experimental assays, synchronized larvae were transferred to treatment-specific vials under controlled conditions.

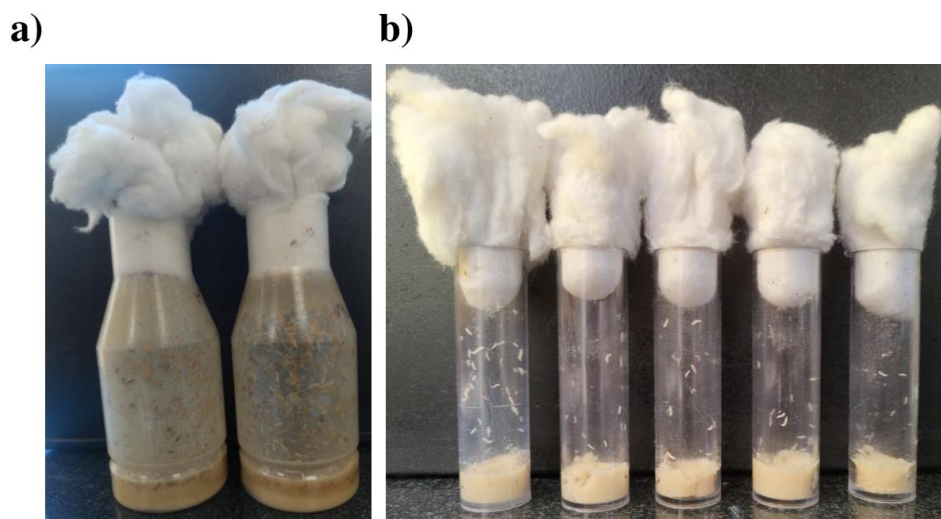


Figure 6.9: Culturing setup for *Drosophila melanogaster* used in experimental studies (a) Stock culture bottles containing standard cornmeal-agar medium used for routine maintenance of *Drosophila melanogaster*, and (b) Experimental vials containing standardized diet medium used for treatment-specific assays.

6.1.10 Acarbose combination alleviates lipid peroxidative damage in H₂O₂-infused *Drosophila* larvae

Next, we assessed the inhibitory effect of acarbose combined with different polymers in *Drosophila* larvae infused with H₂O₂. Larvae treated with H₂O₂ exhibited a 3.56-fold increase in LPO ($p < 0.001$) compared to the control group (Figure 6.10). Treatment with 100% pectin, guar gum, and acarbose reduced LPO by 3.48-fold ($p < 0.001$), 2.99-fold ($p < 0.001$), and 2.72-fold ($p < 0.001$), respectively. However, 100% β -CD did not show a significant reduction in LPO.

When acarbose was combined with selected polymers, significant reductions in LPO were observed, compared to H₂O₂-treated *Drosophila* larvae. At a 25:75 ratio, pectin and guar gum reduced LPO by 2.84-fold ($p < 0.001$) and 3-fold ($p < 0.001$), respectively, while β -CD showed a modest 1.1-fold reduction ($p < 0.05$). Similarly, at a 75:25 ratio, pectin, guar gum, and β -CD decreased LPO by 2.52-fold ($p < 0.001$), 2.84-fold ($p < 0.001$), and 1.46-fold ($p < 0.01$), respectively. The combination of acarbose with pectin, guar gum, and β -CD at 50:50 ratio resulted in a 3.23-fold ($p < 0.001$), 3.45-fold ($p < 0.001$), and 1.60-fold ($p < 0.01$) reduction in LPO. These findings suggest that the 25:75 combination significantly reduces LPO, with the 50:50 ratio showing a substantial reduction in H₂O₂-induced oxidative stress.

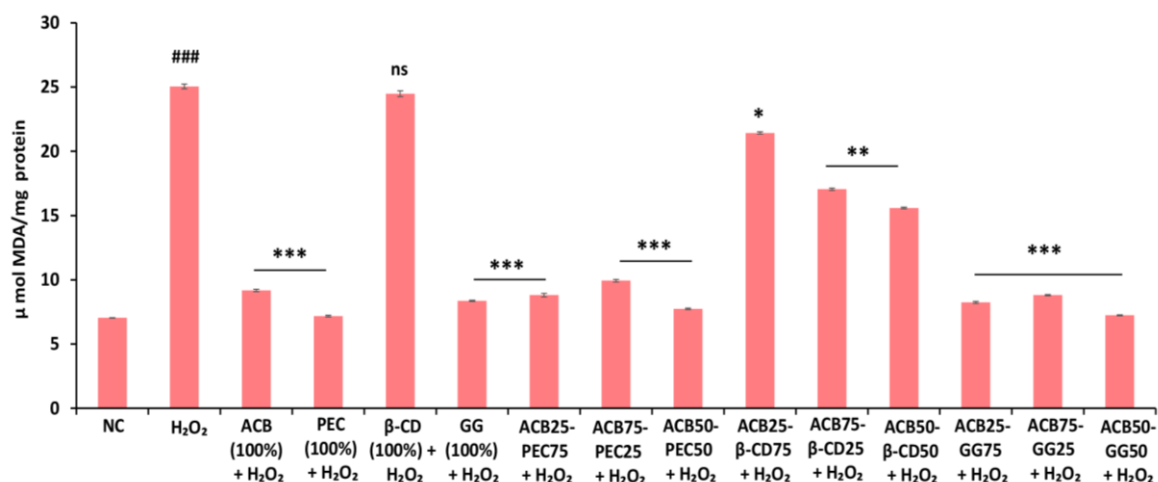


Figure 6.10: Effect of acarbose (ACB), individual polymers (PEC: pectin, β-CD: β-cyclodextrin, GG: guar gum), and their combinations at various ratios (100%, 25:75, 75:25, and 50:50 drug:polymer) on LPO levels in *Drosophila melanogaster* under normal control (NC) and hydrogen peroxide (H₂O₂)-induced oxidative stress conditions. LPO was assessed by measuring MDA content and expressed as μmol MDA/mg protein. Data are presented as mean ± SEM (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ###p < 0.001 compared to NC; *p < 0.05, **p < 0.01, ***p < 0.001 compared to H₂O₂-treated group; ns = non-significant.

6.1.11 Acarbose combination enhances SOD activity in *Drosophila* larvae under oxidative stress

The results shown in Figure 6.11 pertain to the effect of different combinations on the activity of SOD. *Drosophila* larvae treated with H₂O₂ showed a 3.42-fold reduction in SOD activity (p < 0.001) compared to the control group. Treatment with 100% acarbose, pectin, β-CD, and guar gum increased SOD activity by 2.38-fold (p < 0.001), 2.81-fold (p < 0.001), 1.83-fold (p < 0.05), and 2.70-fold (p < 0.001), respectively. While all treatments significantly elevated activity, β-CD had a comparatively lower effect.

The combination of acarbose with pectin, β-CD, and guar gum at a 25:75 ratio increased SOD activity by 2.36-fold (p < 0.001), 1.56-fold (p < 0.05), and 2.16-fold (p < 0.01), respectively, compared to H₂O₂-treated larvae. At a 75:25 ratio, pectin, β-CD, and guar gum increased activity by 2.14-fold (p < 0.01), 1.87-fold (p < 0.05), and 2.38-fold (p < 0.001), respectively. Similarly, the combination of acarbose with pectin, β-CD, and guar gum resulted in increases

of 3-fold ($p < 0.001$), 2-fold ($p < 0.01$), and 2.88-fold ($p < 0.001$), respectively at 50:50 ratio. Overall, the 50:50 combination showed the most substantial enhancement in response to H_2O_2 -induced oxidative stress among all combinations.

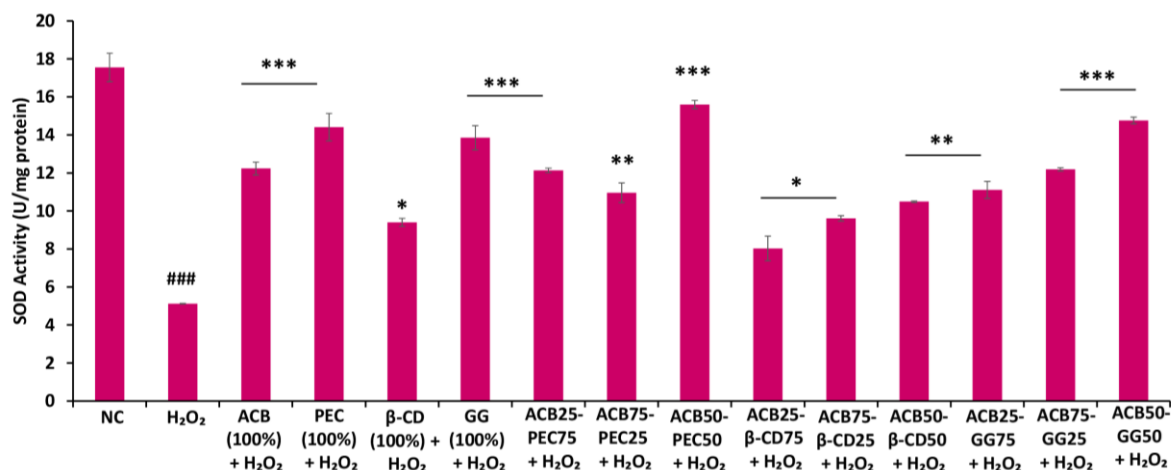


Figure 6.11: Effect of acarbose (ACB), individual polymers (PEC: pectin, β -CD: β -cyclodextrin, GG: guar gum), and their combinations at various ratios (100%, 25:75, 75:25, and 50:50 drug:polymer) on SOD activity in *Drosophila melanogaster* under normal control (NC) and hydrogen peroxide (H_2O_2)-induced oxidative stress conditions. SOD activity was measured in units per mg protein (U/mg protein). Data are presented as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared to NC; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to H_2O_2 -treated group.

6.1.12 Restoration of catalase activity by acarbose-polymer combinations

As indicated in Figure 6.12, *Drosophila* larvae exposed to H_2O_2 exhibited a significant 2.94-fold reduction in catalase activity ($p < 0.001$). Treatment with acarbose, pectin, β -CD, and guar gum at 100% concentration resulted in increases in catalase activity by 2.15-fold ($p < 0.001$), 2.35-fold ($p < 0.001$), 1.56-fold ($p < 0.05$), and 2.34-fold ($p < 0.001$), respectively, compared to H_2O_2 -exposed larvae. Notably, β -CD showed a comparatively lower potential for restoring catalase activity.

Compared to H_2O_2 -treated *Drosophila* larvae, the combination of pectin and guar gum at a 25:75 ratio significantly increased catalase activity by 2.36-fold ($p < 0.001$) and 2-fold ($p < 0.001$), respectively. At the 75:25 ratio, pectin, β -CD, and guar gum increased catalase activity

by 1.86-fold ($p < 0.01$), 1.71-fold ($p < 0.05$), and 1.80-fold ($p < 0.01$), respectively. Furthermore, the combination of acarbose with pectin, β -CD, and guar gum at 50:50 ratio showed the most significant enhancement, with increases of 2.77-fold ($p < 0.001$), 1.86-fold ($p < 0.01$), and 2.51-fold ($p < 0.001$), respectively. Together, these findings indicate that the 50:50 combination provided the greatest enhancement in catalase activity in response to H_2O_2 -induced oxidative stress, compared to all other combinations.

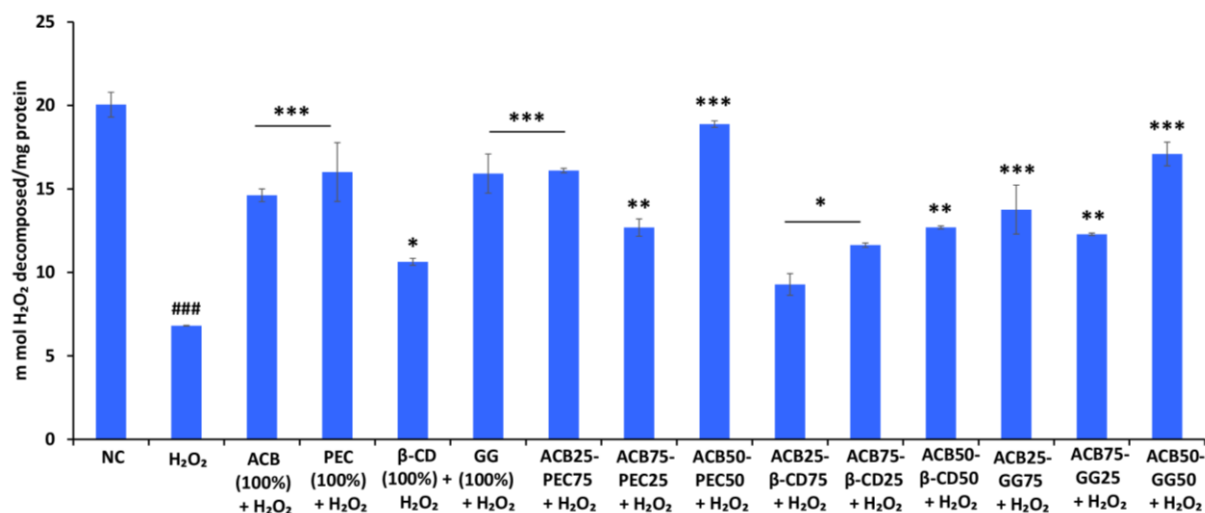


Figure 6.12: Effect of acarbose (ACB), individual polymers (PEC: pectin, β -CD: β -cyclodextrin, GG: guar gum), and their combinations at various ratios (100%, 25:75, 75:25, and 50:50 drug:polymer) on catalase activity in *Drosophila melanogaster* under normal control (NC) and hydrogen peroxide (H_2O_2)-induced oxidative stress conditions. Catalase activity was expressed as mmol H_2O_2 decomposed per mg protein. Data are presented as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared to NC; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to H_2O_2 -treated group.

6.1.13 Reduction of AChE activity by acarbose-polymer combinations

Since AChE contributes to oxidative stress by generating ROS, we evaluated the effect of different treatments on its activity. The results showed that larvae treated with H_2O_2 exhibited a 5.62-fold increase ($p < 0.001$) in AChE activity compared to the normal control group (Figure 6.13). Treatment with acarbose, pectin, β -CD, and guar gum at 100% concentration significantly reduced AChE activity by 3.25-fold ($p < 0.001$), 4-fold ($p < 0.001$), 1.11-fold ($p < 0.05$), and 3.33-fold ($p < 0.001$), respectively, compared to H_2O_2 -treated larvae.

The combination of acarbose with pectin, β -CD, and guar gum at a 25:75 ratio resulted in reductions in AChE activity by 3.17-fold ($p < 0.001$), 1.78-fold ($p < 0.01$), and 3.95-fold ($p < 0.001$), respectively. The 75:25 ratio also significantly reduced AChE activity, with pectin showing a 2.99-fold decrease ($p < 0.001$), β -CD a 2.33-fold decrease ($p < 0.001$), and guar gum a 3.42-fold decrease ($p < 0.001$). Additionally, the 50:50 combination resulted in reductions of 4.10-fold ($p < 0.001$), 2.66-fold ($p < 0.001$), and 4.63-fold ($p < 0.001$), respectively. These findings suggest that the 25:75 combination (except for β -CD) was more effective than the 75:25 ratio. The 50:50 combination exhibited the strongest reduction in AChE activity across all combinations when compared to H_2O_2 -treated larvae.

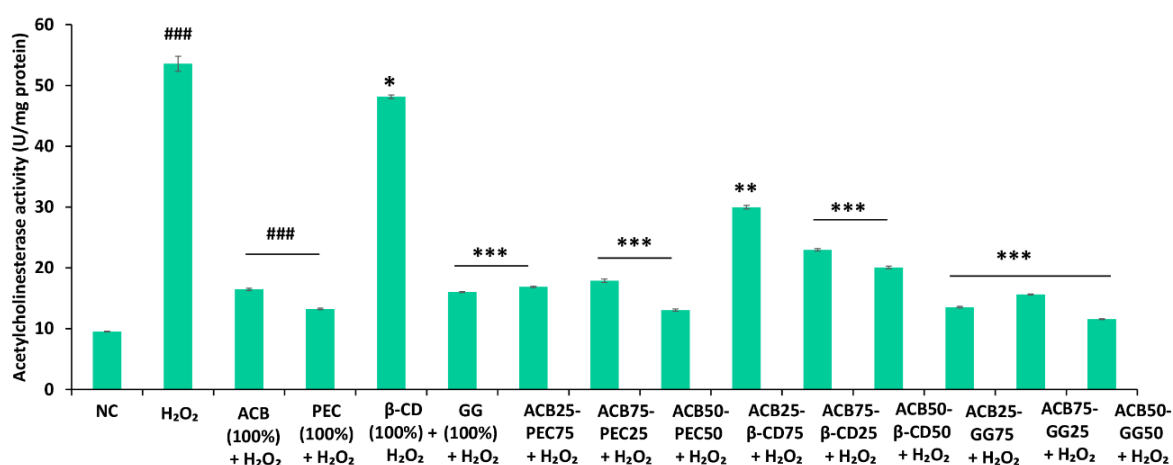


Figure 6.13: Effect of acarbose (ACB), individual polymers (PEC: pectin, β -CD: β -cyclodextrin, GG: guar gum), and their combinations at various ratios (100%, 25:75, 75:25, and 50:50 drug:polymer) on AChE activity in *Drosophila melanogaster* under normal control (NC) and hydrogen peroxide (H_2O_2)-induced oxidative stress conditions. AChE activity was measured in units per mg protein (U/mg protein). Data are presented as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared to NC; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to H_2O_2 -treated group.

6.1.14 Inhibitory effect of acarbose-polymer combinations on Xanthine oxidase activity

Since oxidative stress influences the activity of xanthine oxidase, we evaluated the effect of different treatments on its activity to mitigate oxidative stress. As shown in Figure 6.14, larvae exposed to H_2O_2 exhibited a 5.09-fold increase ($p < 0.001$) in xanthine oxidase activity compared to the normal control group. Treatment with acarbose, pectin, β -CD, and guar gum

at 100% concentration significantly reduced xanthine oxidase activity by 2.39-fold ($p < 0.001$), 2.95-fold ($p < 0.001$), 1.13-fold ($p < 0.05$), and 2.54-fold ($p < 0.001$), respectively, compared to H_2O_2 -treated larvae.

Compared to H_2O_2 -treated larvae, the combination of acarbose with pectin and guar gum at a 25:75 ratio led to a 2.07-fold ($p < 0.01$) reduction in xanthine oxidase activity, whereas β -CD showed a smaller 1.41-fold reduction ($p < 0.05$). In the 75:25 ratio, acarbose with pectin, β -CD, and guar gum reduced xanthine oxidase activity by 1.85-fold ($p < 0.01$), 1.67-fold ($p < 0.01$), and 1.84-fold ($p < 0.01$), respectively. The 50:50 combination of acarbose with pectin, β -CD, and guar gum resulted in reductions of 2.67-fold ($p < 0.001$), 1.83-fold ($p < 0.01$), and 2.62-fold ($p < 0.001$), respectively. In conclusion, the 75:25 combination significantly reduced xanthine oxidase activity (except for β -CD) compared to the 25:75 ratio. Additionally, the 50:50 combination of acarbose with these polymers showed the most substantial reduction in xanthine oxidase activity, across all combinations.

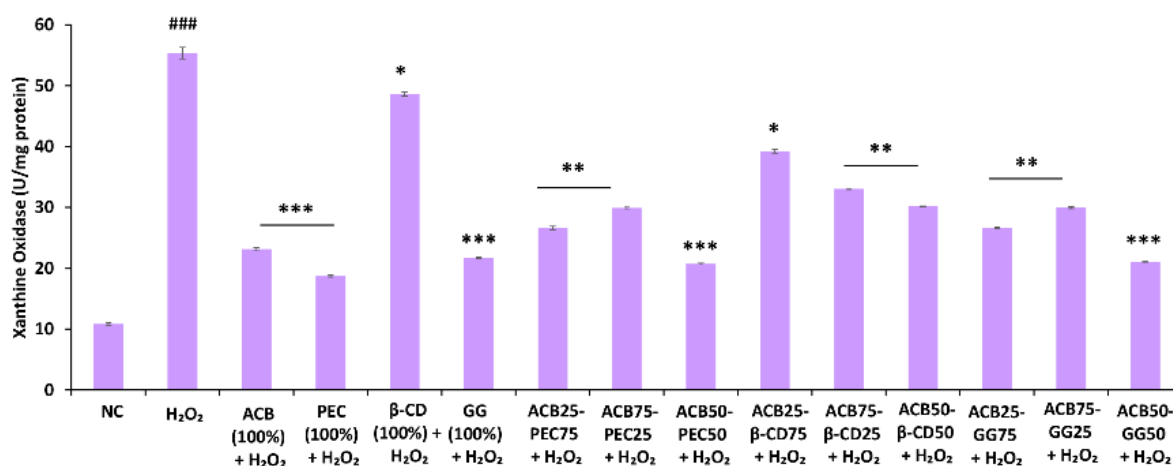


Figure 6.14: Effect of acarbose (ACB), individual polymers (PEC: pectin, β -CD: β -cyclodextrin, GG: guar gum), and their combinations at various ratios (100%, 25:75, 75:25, and 50:50 drug:polymer) on xanthine oxidase in *Drosophila melanogaster* under normal control (NC) and hydrogen peroxide (H_2O_2)-induced oxidative stress conditions. Xanthine oxidase activity was measured in units per mg protein (U/mg protein). Data are presented as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared to NC; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to H_2O_2 -treated group.

6.1.15 Administration of acarbose-polymer combination modulates inflammatory markers

Exposure to H₂O₂ significantly increased the expression of both the pro-inflammatory cytokine TNF- α by 2.79-fold ($p < 0.001$) and the anti-inflammatory cytokine IL-10 by 4.33-fold ($p < 0.001$), compared to the normal control, indicating an oxidative stress-induced inflammatory response (Figure 6.15 a-b). Treatment with acarbose (100%) in the presence of H₂O₂ markedly decreased TNF- α levels to 1.31-fold ($p < 0.01$) and enhanced IL-10 levels to 2.65-fold ($p < 0.01$), demonstrating a strong anti-inflammatory effect by suppressing pro-inflammatory signaling while promoting anti-inflammatory responses. Pectin (100%) combined with H₂O₂ also reduced TNF- α expression to 1.65-fold ($p < 0.001$) and increased IL-10 levels to 3.16-fold ($p < 0.001$), indicating a moderate attenuation of inflammation. Similarly, guar gum (100%) showed a comparable trend, reducing TNF- α to 1.78-fold ($p < 0.001$) and increasing IL-10 to 2.66-fold ($p < 0.01$), reflecting its potential anti-inflammatory properties. In contrast, β -CD (100%) exhibited minimal effect, with only a 1.11-fold reduction in TNF- α and a 1.54-fold increase in IL-10.

The 50:50 combination of acarbose with pectin led to a 1.77-fold reduction in TNF- α ($p < 0.001$) and a relatively higher IL-10 level of 3.74-fold ($p < 0.001$), suggesting effective suppression of pro-inflammatory markers along with a sustained anti-inflammatory response. Conversely, the combination of 50% acarbose with 50% guar gum in the presence of H₂O₂ resulted in a 1.38-fold reduction in TNF- α ($p < 0.001$) and a 3.65-fold increase in IL-10 ($p < 0.001$), indicating a balanced modulation of cytokine signaling. The combination of 50% acarbose with 50% β -CD led to a 1.90-fold reduction in TNF- α and a 2.07-fold increase in IL-10, reflecting a moderate anti-inflammatory effect. Overall, acarbose alone and in combination with pectin effectively mitigated oxidative stress-induced inflammation by lowering TNF- α levels and enhancing IL-10 levels, highlighting their potential synergistic anti-inflammatory properties.

Collectively, the *in vivo* findings align with the *in silico* interaction analysis, which demonstrated favourable molecular compatibility between acarbose and pectin/guar gum. The enhanced biological performance of the 50:50 combinations across oxidative stress and inflammatory parameters suggests that optimal drug-polymer interaction may enhance functional stability and bioactivity under stress conditions. These results indicate that

molecular interaction strength may contribute to improved therapeutic potential in oxidative stress-associated conditions.

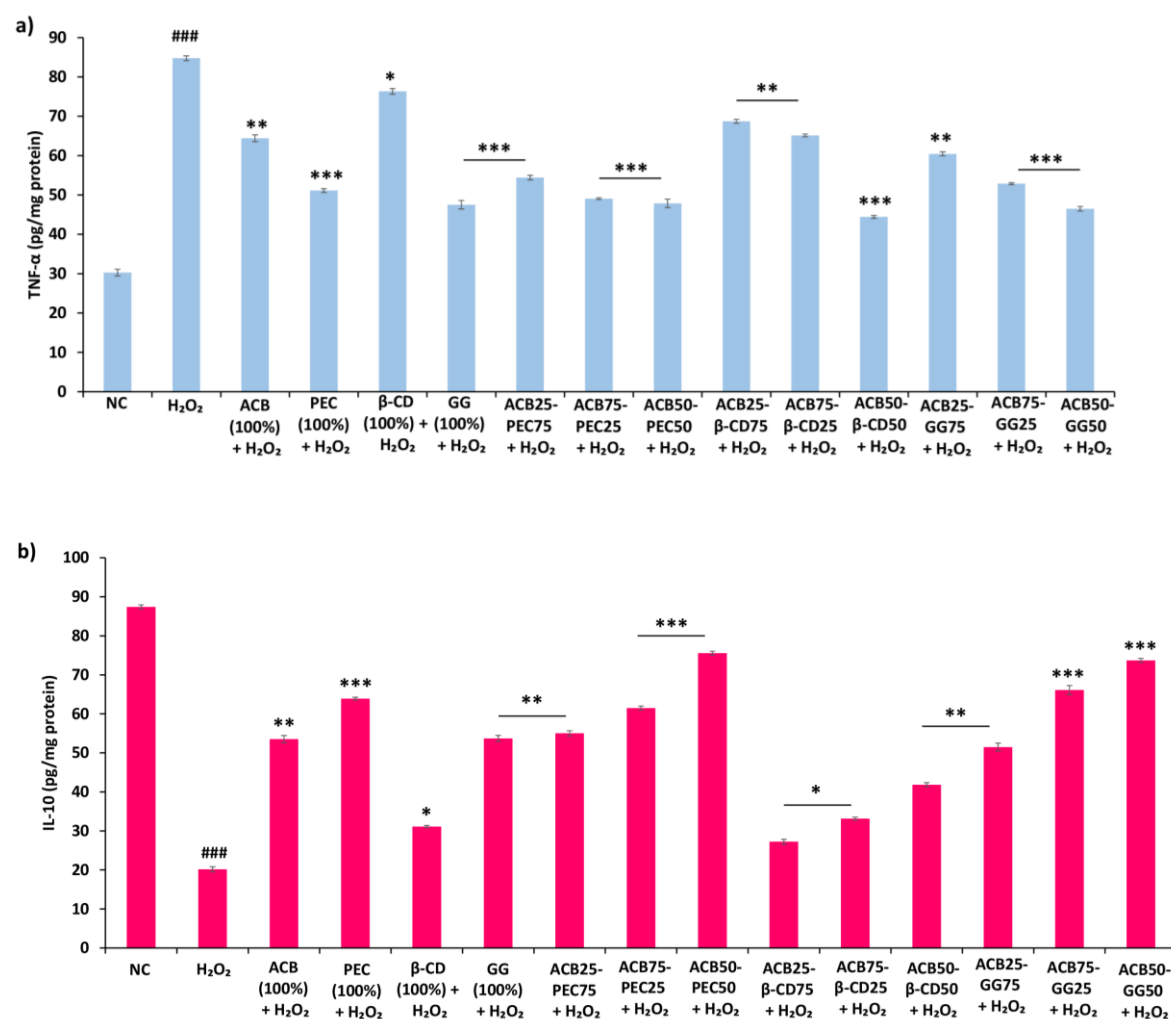


Figure 6.15: Effect of acarbose (ACB), individual polymers (PEC: pectin, β-CD: β-cyclodextrin, GG: guar gum), and their combinations at various ratios (100%, 25:75, 75:25, and 50:50 drug:polymer) on inflammatory markers, **(a)** TNF-α and **(b)** IL-10 in *Drosophila melanogaster* under normal control (NC) and hydrogen peroxide (H₂O₂)-induced oxidative stress conditions. TNF-α and IL-10 activities were measured in picograms per milligram of protein (pg/mg protein). Data are presented as mean ± SEM (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ####p < 0.001 compared to NC; *p < 0.05, **p < 0.01, ***p < 0.001 compared to H₂O₂-treated group.

6.1.16 Rationale for selection of guar gum for nanoformulation development

The selection of guar gum for nanoformulation development was based on a comprehensive and integrated evaluation of *in silico*, *in vitro*, and *in vivo Drosophila* findings, along with therapeutic and translational considerations. Molecular docking and dynamics simulation demonstrated that β -cyclodextrin exhibited the strongest binding affinity with acarbose, whereas guar gum showed moderate binding energy with stable RMSD profiles over 400 ns. The moderate interaction strength observed with guar gum is considered advantageous for controlled-release systems, as excessively strong binding may restrict drug availability, while weaker interactions may compromise stability. Thus, guar gum provided an optimal balance between structural stability and functional drug release.

Although both pectin and guar gum demonstrated substantial radical-scavenging activity in non-enzymatic antioxidant assays and effectively mitigated oxidative stress in the *Drosophila melanogaster* model, guar gum exhibited comparatively more consistent and robust biological responses. Notably, guar gum treatment resulted in pronounced restoration of endogenous antioxidant enzymes, significant reduction in oxidative lipid damage, marked suppression of cholinergic and ROS-generating enzymatic activities, and balanced modulation of inflammatory cytokines. In contrast, β -CD showed comparatively weaker antioxidant and anti-inflammatory effects despite its higher docking affinity, suggesting that stronger complexation did not necessarily translate into superior biological performance.

Importantly, guar gum possesses intrinsic antidiabetic properties, including delayed carbohydrate absorption and modulation of postprandial glycemia, which complement the α -glucosidase inhibitory activity of acarbose, thereby supporting a synergistic therapeutic approach. Furthermore, its hydrophilic, swelling, and matrix-forming characteristics make it particularly suitable for sustained-release nanoformulation development. India ranks among the leading global producers of guar gum; therefore, its incorporation into pharmaceutical formulations represents a cost-effective, scalable, and strategically viable approach for the value-added utilization of indigenous agricultural resources. Collectively, considering its optimal drug-polymer interaction profile, consistent antioxidant and anti-inflammatory efficacy, antidiabetic synergy, controlled-release suitability, and economic relevance, guar gum was selected over pectin and β -CD for the preparation of the spray-dried nanoformulation.

6.2 Preparation of spray dried formulation

In this setup, a liquid or slurry feed is atomized into fine droplets and exposed to a stream of hot air, causing rapid solvent evaporation (Figure 6.16a-b). The resulting dry particles are carried with the airflow into the cyclone chamber, where centrifugal force separates the solid particles from the air. The fine powder collects in the bottom container, while the air exits through the top outlet.

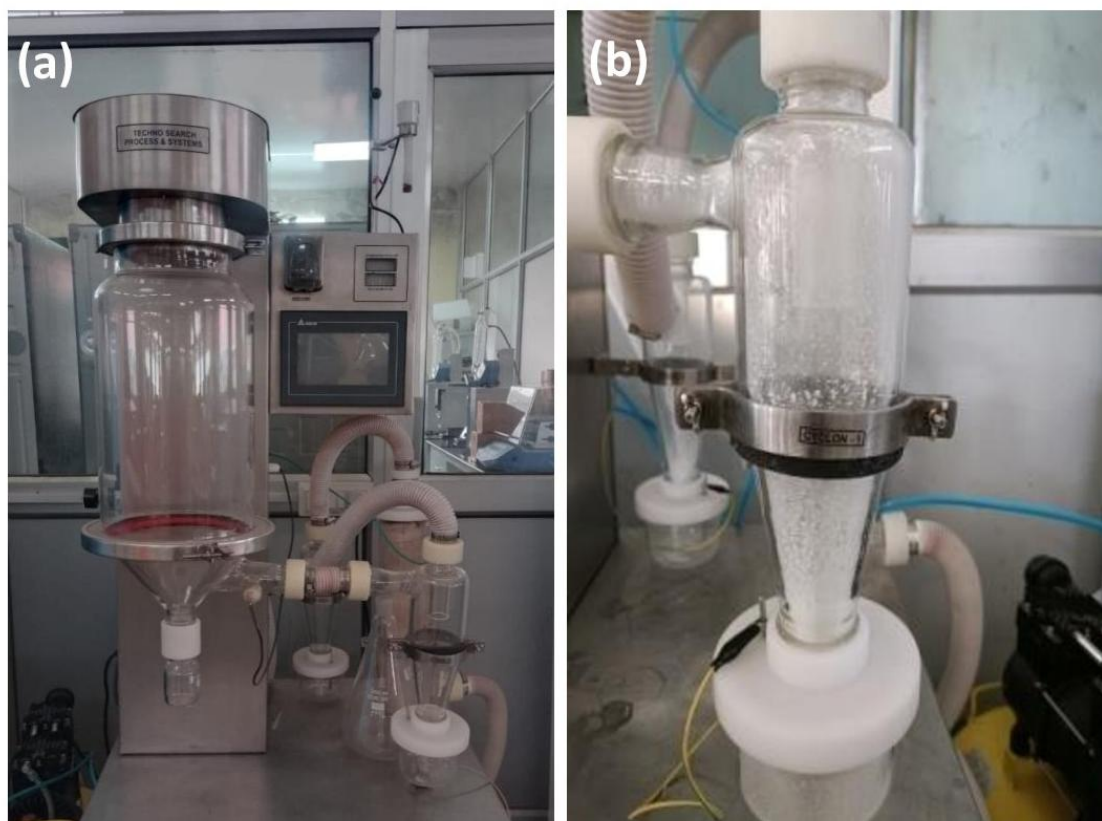


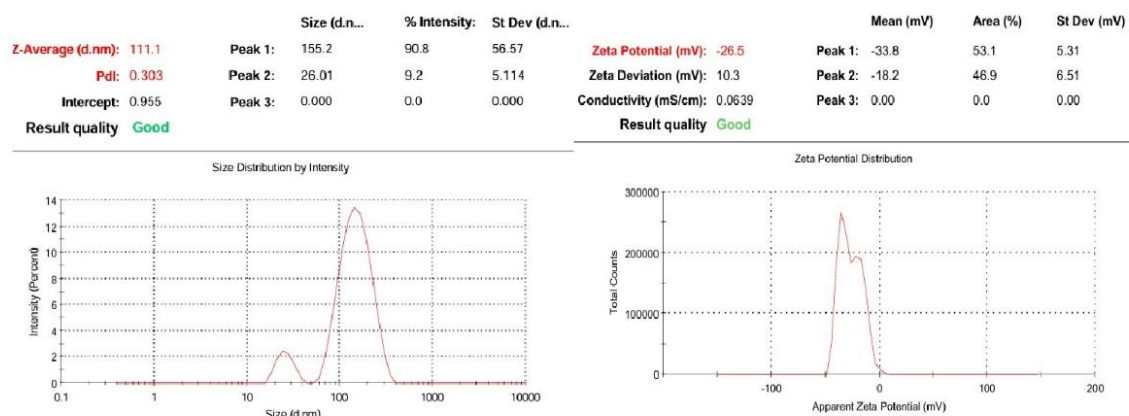
Figure 6.16: (a) Schematic representation of the spray drying apparatus employed for formulation preparation; (b) Cyclone collector depicting the collection of the spray-dried formulation post-processing.

6.2.1 Particle size and zeta potential

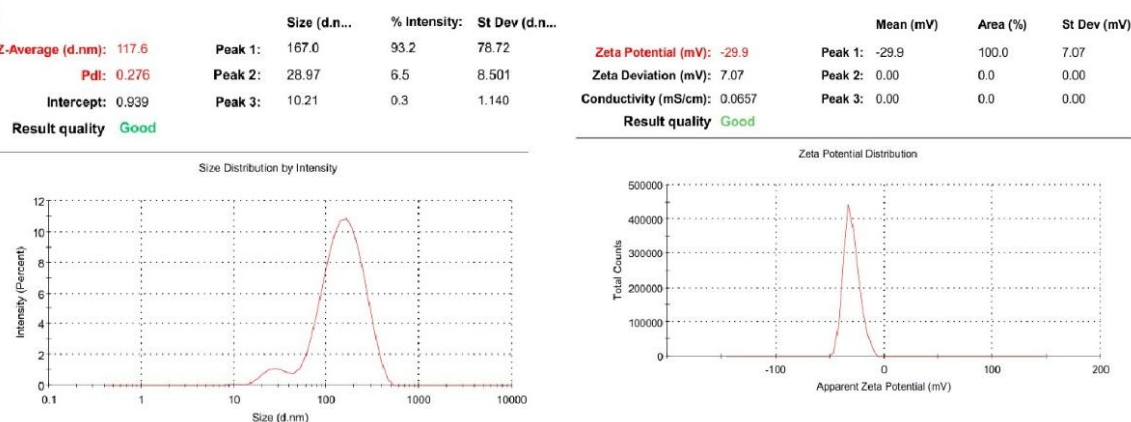
Solid dispersion-based nanoformulations of acarbose and guar gum were successfully developed using varying drug-to-polymer ratios and were characterized for particle size and zeta potential. The particle size and surface charge of the nanoformulations were significantly influenced by the polymer content. AEGN-1 exhibited the largest particle size of 111.1 nm with a PDI value of 0.303 and a zeta potential of -26.5 mV, likely due to the lower polymer content

providing limited steric stabilization. In contrast, AEGN-2 showed a particle size of 117.6 nm, a PDI value of 0.276, and the most negative zeta potential of -29.9 mV, suggesting enhanced colloidal stability. AEGN-3 exhibited the smallest particle size of 121.4 nm, a PDI value of 0.341, and a zeta potential of -27.2 mV, indicating good electrostatic stability compared to AEGN-1 and AEGN-2. These findings underscore the impact of polymer concentration on particle size distribution and colloidal stability, as shown in Figure 6.17A to C.

(A)



(B)



(C)

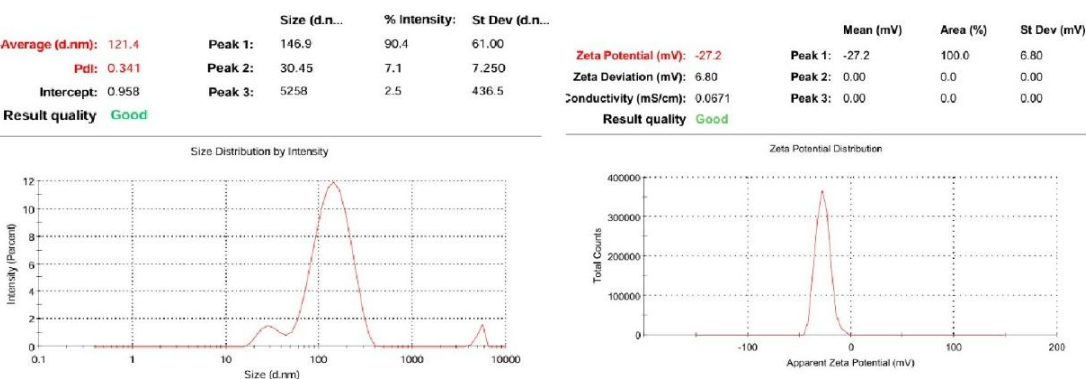


Figure 6:17: Particle size, PDI, and zeta potential of (a) AEGN-1, (b) AEGN-2, and (c) AEGN-3.

6.2.2 SEM analysis of morphological characteristics of AEGN formulations

The morphology and particle shape of pure acarbose, guar gum, solid dispersion, and physical mixtures were examined using SEM (Figure 6.18 a-h). The SEM image of pure guar gum displayed an irregular surface with a rough and porous texture, indicative of its fibrous structure. The presence of small dots and nodules on the surface may be due to its natural polysaccharide characteristics. In contrast, the pure acarbose showed a smooth and dense surface with a semi-crystalline appearance, characterized by larger and more uniform particle shapes with distinct edges.

The developed formulations exhibited significant morphological alterations, predominantly attributed to the spray-drying process. The SEM images revealed spherical particles, suggesting successful encapsulation of acarbose within the guar gum matrix. These spherical structures result from the atomization and rapid drying during the spray-drying process. The observed white-coloured patches on the solid dispersion particles could be attributed to surface crystallization of acarbose or exposure to environmental humidity during sample preparation. However, in the physical mixture, there was no evidence of encapsulation of acarbose within the guar gum polymer. The SEM images show that the crystalline acarbose particles adhered to the rough surface of guar gum, indicating a simple mechanical blend rather than molecular interaction or dispersion.

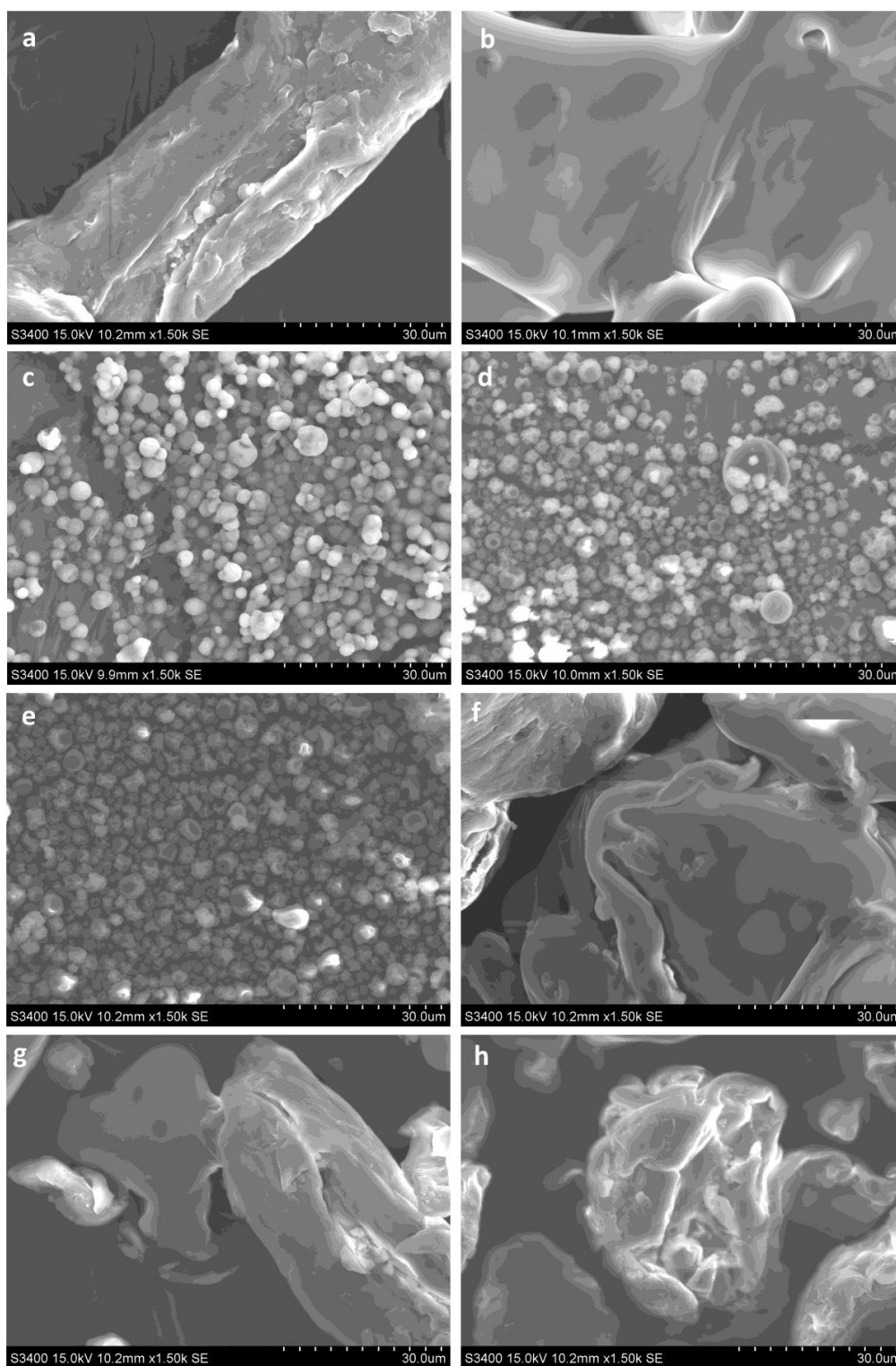


Figure 6.18: Scanning electron microscopy (SEM) images showing the surface morphology of (a) guar gum, (b) acarbose, (c) AEGN-1, (d) AEGN-2, (e) AEGN-3, (f) PM-1, (g) PM-2, and (h) PM-3 at 1500 $\times$ magnification. Scale bars represent 30 μ m. AEGN: Acarbose-encapsulated guar gum nanoformulation; PM: Physical mixture.

6.2.3 FTIR analysis of molecular interactions in AEGN formulations

The FTIR spectrum of pure acarbose revealed characteristic peaks indicative of its functional groups. The broad O-H stretching band was observed at 3413.76 cm^{-1} , that reflects the presence of hydroxyl groups within the acarbose structure. The C-H stretching vibration appeared at 2927.47 cm^{-1} . Additionally, the medium C-H bending was observed at 1353.69 cm^{-1} , and C-O stretching vibrations appeared at 1037.98 cm^{-1} . Although acarbose contains an N-H amide group between 3310 to 3360 cm^{-1} , this peak was not distinctly observed due to overlap with the broad O-H stretch in the FTIR spectrum. In contrast, the FTIR spectrum of pure guar gum revealed broad absorption bands around 3470.28 cm^{-1} , 3547.84 cm^{-1} , and 3414.69 cm^{-1} , corresponding to O-H stretching vibrations, which are characteristic of polysaccharides. The C-H stretching band appeared at 2925.56 cm^{-1} , while C-H bending was observed at 1384.33 cm^{-1} and 1353.33 cm^{-1} . Additionally, C-O-C stretching bands were present at 1023.50 cm^{-1} and 1096.26 cm^{-1} , and hydroxyl bending was seen at 1638.01 cm^{-1} and 1617.58 cm^{-1} , further confirming the structural features of guar gum.

Furthermore, the FTIR spectra of the AEGNs revealed the characteristic peaks of both acarbose and guar gum. The presence of C=C stretching, indicative of acarbose, was detected at 1638.28 cm^{-1} , 1638.21 cm^{-1} , and 1637.95 cm^{-1} , along with an additional peak at 1617.71 cm^{-1} , 1617.89 cm^{-1} , and 1618.66 cm^{-1} . C-H bending vibrations were also observed at 1353.33 cm^{-1} , 1353.15 cm^{-1} , and 1352.81 cm^{-1} , as well as at 1383.77 cm^{-1} , 1381.93 cm^{-1} , and 1382.69 cm^{-1} , revealing the encapsulation of acarbose within the guar gum matrix. Similarly, O-H stretching peaks appeared at 3412.82 cm^{-1} , 3411.27 cm^{-1} , and 3412.84 cm^{-1} , while C-H stretching was detected at 2928.74 cm^{-1} , 2926.0 cm^{-1} , and 2927.25 cm^{-1} . the presence of guar gum in the formulations was further confirmed by the C-O-C stretching peaks at 1029.64 cm^{-1} , 1027.60 cm^{-1} , and 1026.40 cm^{-1} . Notably, the encapsulation of acarbose into guar gum resulted in the masking of some characteristic peaks of acarbose, particularly the O-H and C-H stretching bands at 3413.76 cm^{-1} and 2927.47 cm^{-1} , indicating a successful interaction between the drug and the polymer during the spray-drying process. In contrast, the FTIR spectra of the physical mixtures (PM-1, PM-2, and PM-3) were similar to those of the pure compounds, showing no significant shifts or interactions between acarbose and guar gum (Figure 6.19). This suggests that in the physical mixtures, acarbose and guar gum simply coexist without forming any significant molecular interactions or complexation.

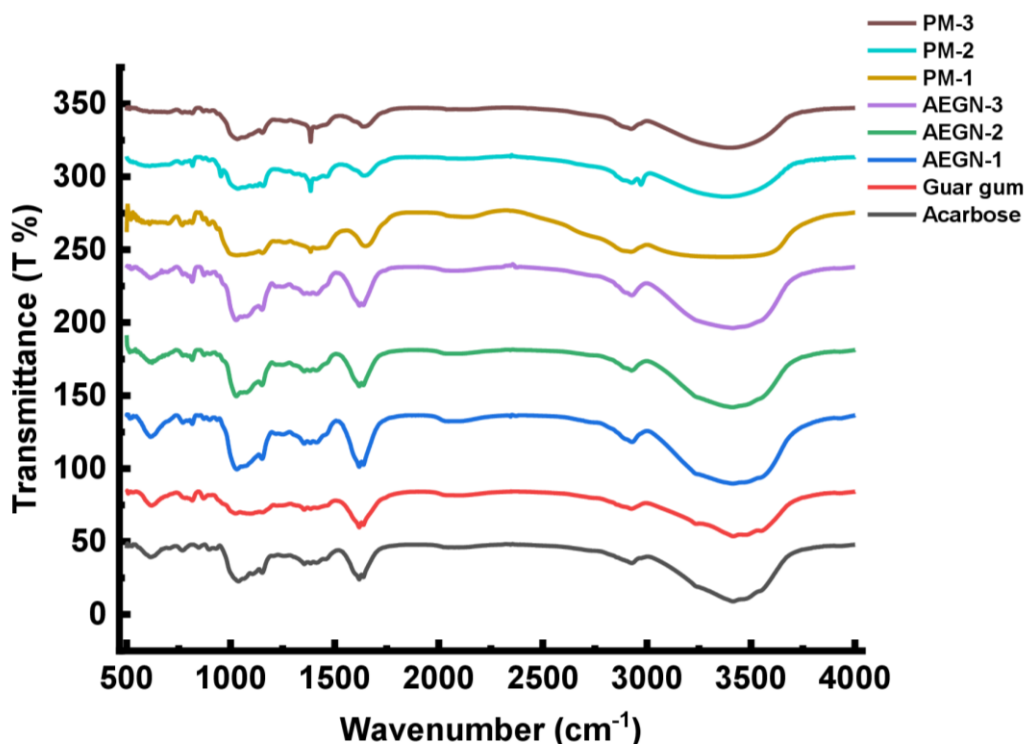


Figure 6.19: FTIR spectra of various compounds including acarbose, guar gum, AEGN-1, AEGN-2, AEGN-3, PM-1, PM-2, and PM-3, showing characteristic functional group vibrations and potential interactions in acarbose-encapsulated guar gum nanoformulation (AEGN) and physical mixture (PM).

6.2.4 pXRD analysis of acarbose crystallinity in AEGN formulations

pXRD analysis reveals distinct differences in the solid-state properties of pure compounds, AEGN formulations, and physical mixtures. Pure acarbose exhibits a sharp and intense peak at $2\theta \approx 19.89^\circ$, indicative of its crystalline nature. Likewise, guar gum displays a characteristic peak at $2\theta \approx 20.107^\circ$, reflecting its semi-crystalline structure. These defined diffraction peaks serve as references for assessing changes in crystallinity upon formulation.

The AEGN formulations (AEGN-1, AEGN-2, and AEGN-3), however, exhibit broad and diffused halos instead of sharp crystalline peaks. Notably, the characteristic peaks of both acarbose and guar gum are absent in all three formulations. This loss of distinct diffraction features indicates a transition from a crystalline to an amorphous state. Such a transformation suggests that acarbose is molecularly dispersed within the guar gum matrix, resulting in successful encapsulation. The amorphous nature of the formulations confirms strong intermolecular interactions and disruption of the ordered lattice structure, which may enhance the drug's solubility and bioavailability.

In contrast, the physical mixtures (PM-1, PM-2, and PM-3) retain discernible diffraction peaks in the same 2θ region. These peaks are broader and exhibit reduced intensity compared to those of the pure components, indicating partial preservation of crystalline structure. Furthermore, among the physical mixtures, PM-3 shows the highest intensity, followed by PM-2 and PM-1, suggesting varying degrees of crystallinity retention depending on component proportion and mixing. Overall, the absence of characteristic crystalline peaks in the AEGN formulations, alongside the persistence of these peaks in the physical mixtures, provides strong evidence that acarbose has been successfully encapsulated within the guar gum matrix, as depicted in Figure 6.20. This encapsulation results in a significant reduction in crystallinity, confirming the conversion to an amorphous system through formulation processing.

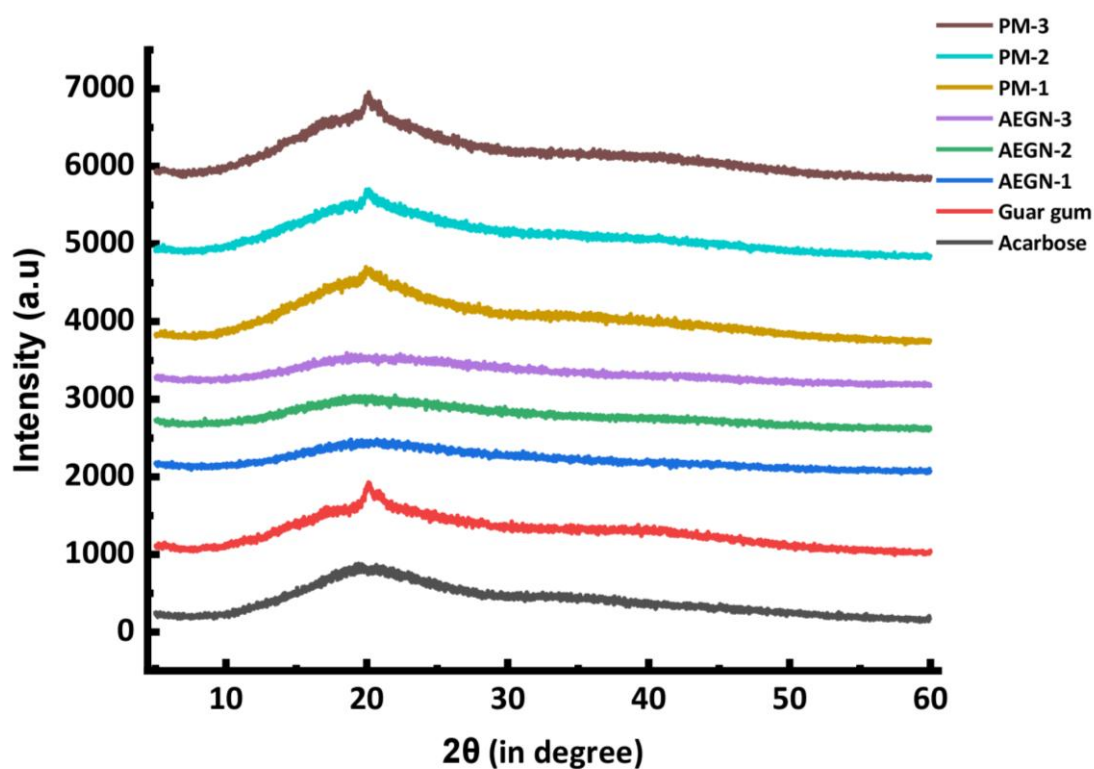


Figure 6.20: pXRD patterns of various compounds including acarbose, guar gum, AEGN-1, AEGN-2, AEGN-3, PM-1, PM-2, and PM-3, showing characteristic diffraction peaks and changes in crystallinity in acarbose-encapsulated guar gum nanoformulation (AEGN) and physical mixture (PM).

6.2.5 Effect of polymer ratio on drug content

AEGN-1 demonstrated the highest drug content of $81.83 \pm 0.76\%$, followed by $48.99 \pm 0.41\%$ for AEGN-2 and $44.50 \pm 0.36\%$ for AEGN-3, indicating a progressive decline in drug content

with increasing polymer proportion. These results suggest that higher polymer ratios reduce the relative drug content, while lower drug-to-polymer ratios enhance polymer encapsulation efficiency, allowing greater drug incorporation at lower drug concentrations.

6.2.6 Effect of drug-to-polymer ratio on drug entrapment efficiency

The drug entrapment efficiency of the nanoformulations depended on the drug-to-polymer ratio. Increasing polymer content led to higher entrapment efficiencies, with AEGN-1, AEGN-2, and AEGN-3 achieving $31.82 \pm 0.32\%$, $58.99 \pm 0.39\%$, and $84.24 \pm 0.48\%$, respectively. This trend suggests that higher polymer ratios promote more effective entrapment of acarbose within the solid dispersion matrix by forming a denser and more cohesive structure, thereby enhancing drug entrapment.

6.2.7 Sustained *in vitro* release of acarbose from guar gum-based AEGN formulations

The cumulative release profiles of acarbose and acarbose-loaded guar gum nanoformulations (AEGN-1, AEGN-2, and AEGN-3) were evaluated in phosphate buffer (pH 6.8) over 24 hours to simulate intestinal conditions (Figure 6.21). Pure acarbose exhibited rapid release, with $86.81 \pm 0.56\%$ released within 6 hours and $93.17 \pm 0.12\%$ by 8 hours, consistent with its immediate-release characteristics. In contrast, the AEGN formulations demonstrated a sustained release pattern dependent on guar gum concentration. AEGN-1 released $78.47 \pm 0.82\%$ at 6 hours and $98.41 \pm 0.82\%$ by 24 hours, whereas AEGN-2 showed a more moderate release, with $58.10 \pm 0.97\%$ at 6 hours and $99.05 \pm 0.56\%$ at 24 hours.

Notably, AEGN-3 exhibited the slowest release, with $37.60 \pm 0.63\%$ at 6 hours and $86.19 \pm 1.66\%$ at 24 hours, indicating effective control of drug release due to increased polymer matrix density. These findings suggest that increasing the guar gum content effectively retards acarbose release by forming a stronger gel barrier that hinders drug diffusion. Such sustained release behaviour may enhance the therapeutic performance of acarbose by maintaining prolonged drug concentrations in the intestinal environment and potentially reducing dosing frequency.

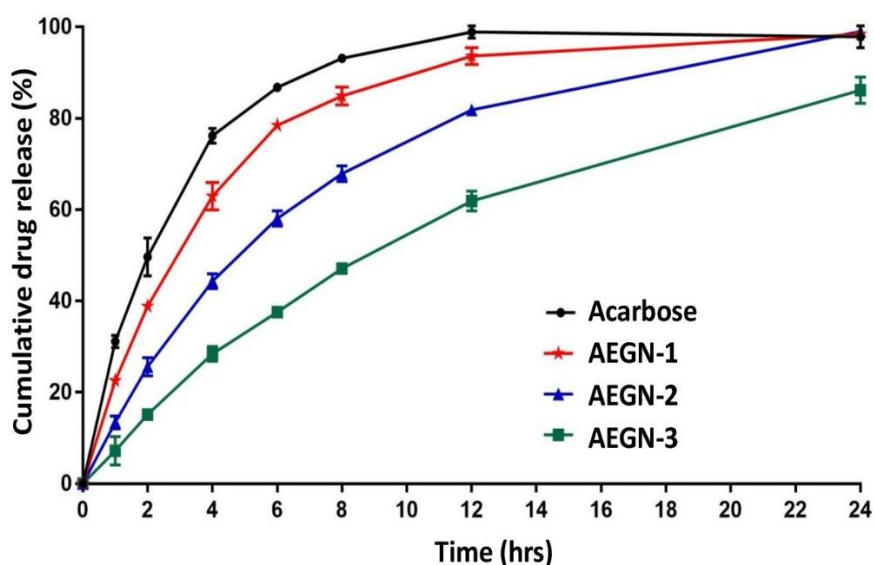


Figure 6.21: *In vitro* cumulative drug release profiles of acarbose and AEGN formulations at pH 6.8. Cumulative % drug release of pure acarbose and acarbose-loaded guar gum nanoformulations (AEGN-1, AEGN-2, and AEGN-3) was assessed in phosphate buffer (pH 6.8) over 24 hours using a dialysis method. Data are expressed as mean $\pm$ SEM (n = 3).

6.2.8 Stability assessment of AEGN formulations

All formulations exhibited minimal variations in physicochemical parameters, indicating good structural integrity and colloidal stability. The drug release profiles remained consistent, suggesting the retention of sustained release characteristics throughout the study duration. Among the tested formulations, AEGN-3 demonstrated superior stability, which may be attributed to its higher polymer concentration, providing a more robust matrix and enhanced protection against environmental stress. These results indicate that the nanoformulations are physically and chemically stable, supporting their potential for long-term pharmaceutical application.

6.3 *In vitro* results

6.3.1 DPPH radical scavenging activity of AEGN

The antioxidant potential of the developed formulations was assessed using the DPPH radical scavenging assay, with L-ascorbic acid used as the reference standard. As illustrated in the figure 6.22, L-ascorbic acid exhibited the strongest radical scavenging activity with the lowest IC₅₀ value of 8.21 ± 0.37 μ g/mL. In contrast, acarbose indicated lower antioxidant potential

with a higher IC_{50} value of $92.74 \pm 0.31 \mu\text{g/mL}$, while guar gum demonstrated moderate antioxidant activity with an IC_{50} value of $51.63 \pm 0.20 \mu\text{g/mL}$.

All three formulations demonstrated improved DPPH scavenging activity compared to acarbose and guar gum alone. Among them, AEGN-3 displayed the highest antioxidant activity with an IC_{50} value of $20.53 \pm 0.45 \mu\text{g/mL}$, followed by AEGN-2 at $25.58 \pm 0.20 \mu\text{g/mL}$ and AEGN-1 at $48.65 \pm 0.41 \mu\text{g/mL}$. These findings suggest that the formulations, particularly AEGN-3, possess significant free radical scavenging potential and may serve as promising candidates for antioxidant-based therapeutic applications.

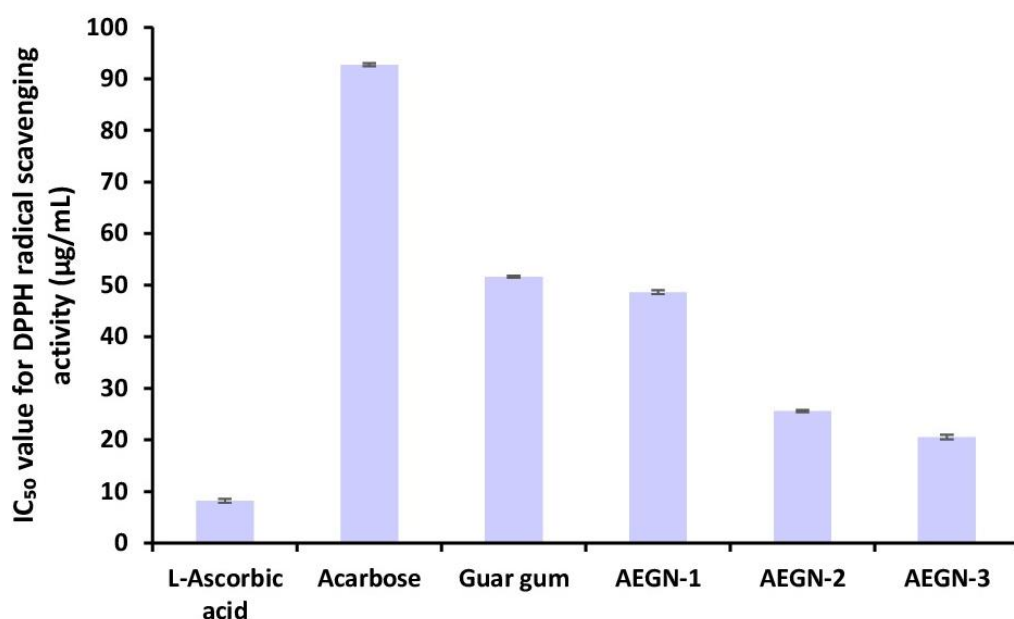


Figure 6.22: IC_{50} values for DPPH radical scavenging activity of L-ascorbic acid, acarbose, guar gum, and AEGN formulations ($\mu\text{g/mL}$). Values are presented as mean $\pm$ SEM ($n = 3$).

6.3.2 ABTS radical scavenging activity of AEGN

The antioxidant potential of the developed formulations was evaluated using the ABTS radical scavenging assay, with Trolox serving as the reference standard. Among the tested compounds, Trolox exhibited the highest antioxidant activity, with the lowest IC_{50} value of $16.61 \pm 0.29 \mu\text{g/mL}$. AEGN-3 demonstrated the strongest radical scavenging effect among the formulations, with an IC_{50} of $18.83 \pm 0.19 \mu\text{g/mL}$, closely approaching that of Trolox. AEGN-2 also showed considerable activity, with an IC_{50} of $32.07 \pm 0.13 \mu\text{g/mL}$, followed by AEGN-1 at $40.6 \pm 0.36 \mu\text{g/mL}$. In comparison, guar gum and acarbose exhibited relatively lower antioxidant potential, with IC_{50} values of $47.3 \pm 0.26 \mu\text{g/mL}$ and $55.2 \pm 0.36 \mu\text{g/mL}$,

respectively. These results suggest that the developed formulations, particularly AEGN-3, possess significant antioxidant activity, compared to both guar gum and acarbose (Figure 6.23).

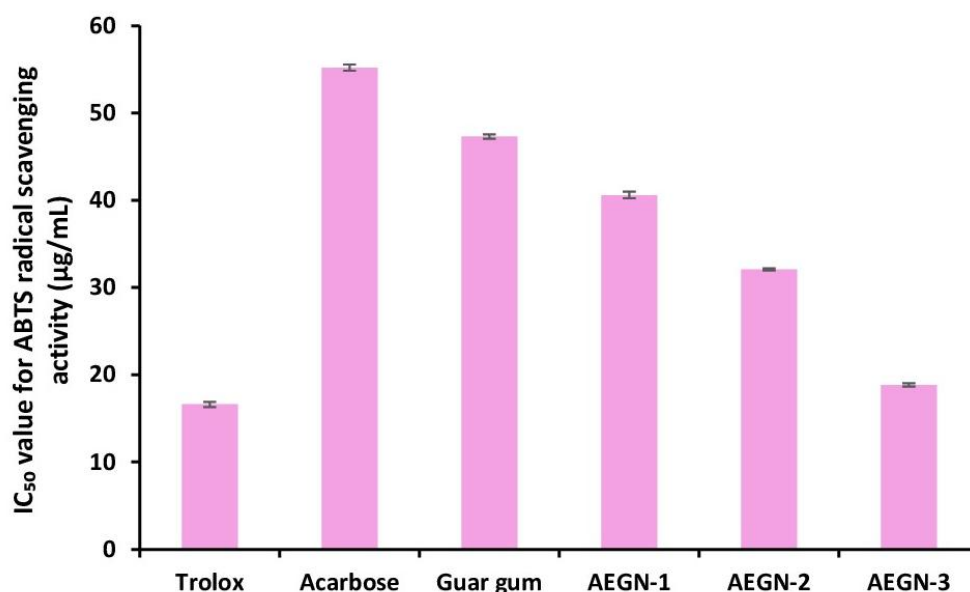


Figure 6.23: IC₅₀ values for ABTS radical scavenging activity of Trolox, acarbose, guar gum, and AEGN formulations (µg/mL). Values are presented as mean ± SEM (n = 3).

6.3.3 Fe²⁺ chelation activity of AEGN

The metal chelation potential of the developed formulations was assessed using EDTA as the reference standard. As illustrated in Figure 6.24, EDTA exhibited the highest metal chelation activity with an IC₅₀ value of 8.33 ± 0.26 µg/mL. In comparison, acarbose and guar gum showed reduced metal chelation activity, with higher IC₅₀ values of 46.93 ± 0.21 µg/mL and 73.08 ± 0.36 µg/mL, respectively, where acarbose demonstrated better activity than guar gum.

All three formulations demonstrated improved metal chelating capacity compared to the individual compounds. Among them, AEGN-3 showed the strongest activity with a low IC₅₀ value of 11.56 ± 0.21 µg/mL, followed by AEGN-2 at 17.88 ± 0.21 µg/mL and AEGN-1 at 38.75 ± 0.27 µg/mL. These results suggest that the formulations effectively enhance metal chelation activity, contributing to their overall antioxidant potential.

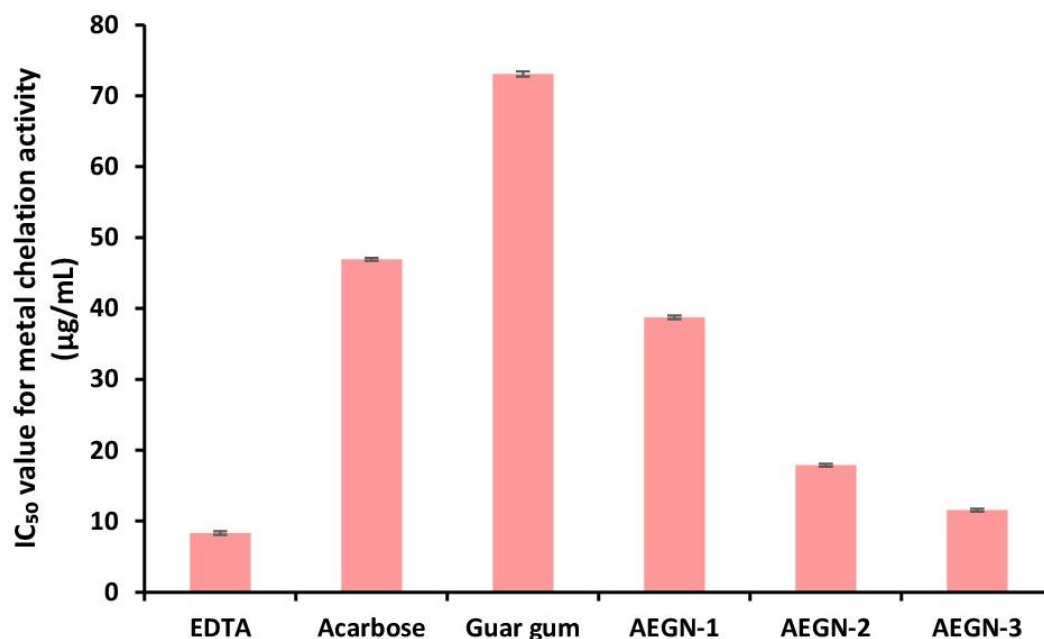


Figure 6.24: IC₅₀ values for metal chelation activity of EDTA, acarbose, guar gum, and AEGN formulations (µg/mL). Values are presented as mean ± SEM (n = 3).

6.3.4 Nitric oxide radical scavenging activity of AEGN

The nitric oxide radical scavenging potential of the formulations was evaluated using Vitamin C as the standard antioxidant. The results showed that vitamin C exhibited the highest activity with an IC₅₀ value of 7.87 ± 0.28 µg/mL, among other tested compounds. In contrast, acarbose and guar gum displayed considerably lower antioxidant potential, with IC₅₀ values of 46.93 ± 0.21 µg/mL and 73.08 ± 0.36 µg/mL, respectively.

Among the formulations, AEGN-3 demonstrated the strongest nitric oxide scavenging activity, showing a low IC₅₀ value of 11.56 ± 0.21 µg/mL, followed by AEGN-2 at 17.88 ± 0.21 µg/mL. AEGN-1 exhibited moderate activity with an IC₅₀ value of 38.75 ± 0.27 µg/mL. These results indicate that the formulations, particularly AEGN-2 and AEGN-3, possess enhanced nitric oxide radical scavenging capacity compared to acarbose and guar gum (Figure 6.25), suggesting their potential as effective antioxidant agents.

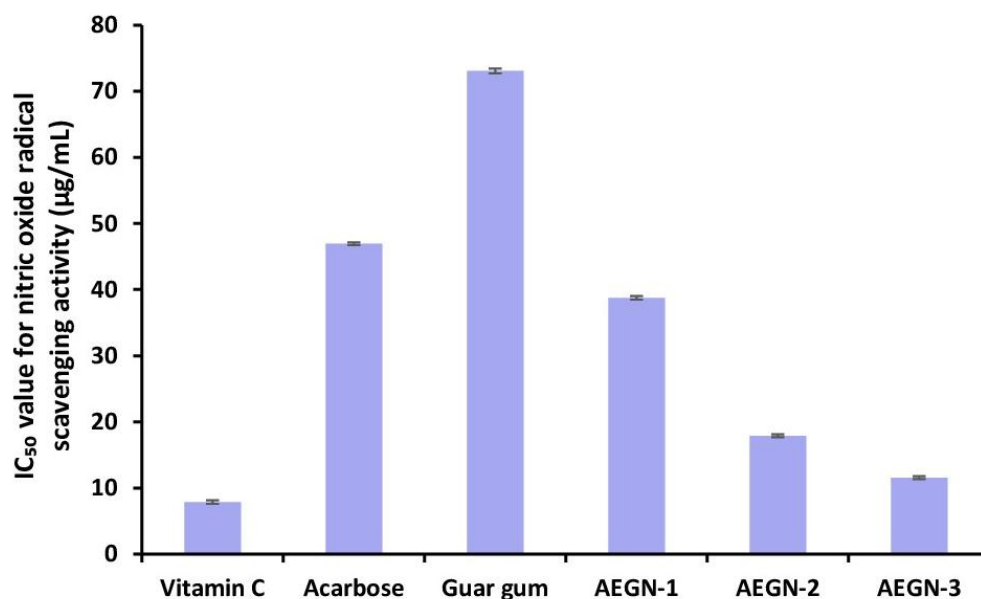


Figure 6.25: IC₅₀ values for nitric oxide radical scavenging activity of Vitamin C, acarbose, guar gum, and AEGN formulations (µg/mL). Values are presented as mean ± SEM (n = 3).

6.3.5 α -amylase inhibitory activity of AEGN

The α -amylase inhibitory potential of the developed formulations was evaluated, with acarbose used as the reference standard. As shown in the figure 6.26, acarbose exhibited the strongest alpha-amylase inhibition with the lowest IC₅₀ value of 6.43 ± 0.32 µg/mL. In contrast, guar gum demonstrated weaker inhibition, with an IC₅₀ value of 44.87 ± 0.29 µg/mL. Among the formulations, AEGN-3 displayed the highest alpha-amylase inhibitory activity, with an IC₅₀ value of 12.86 ± 0.18 µg/mL, followed by AEGN-2 at 20.36 ± 0.23 µg/mL and AEGN-1 at 26.65 ± 0.14 µg/mL. These results suggest that the formulations, particularly AEGN-3, exhibit significant alpha-amylase inhibitory potential and could be considered for the development of anti-diabetic agents targeting carbohydrate digestion.

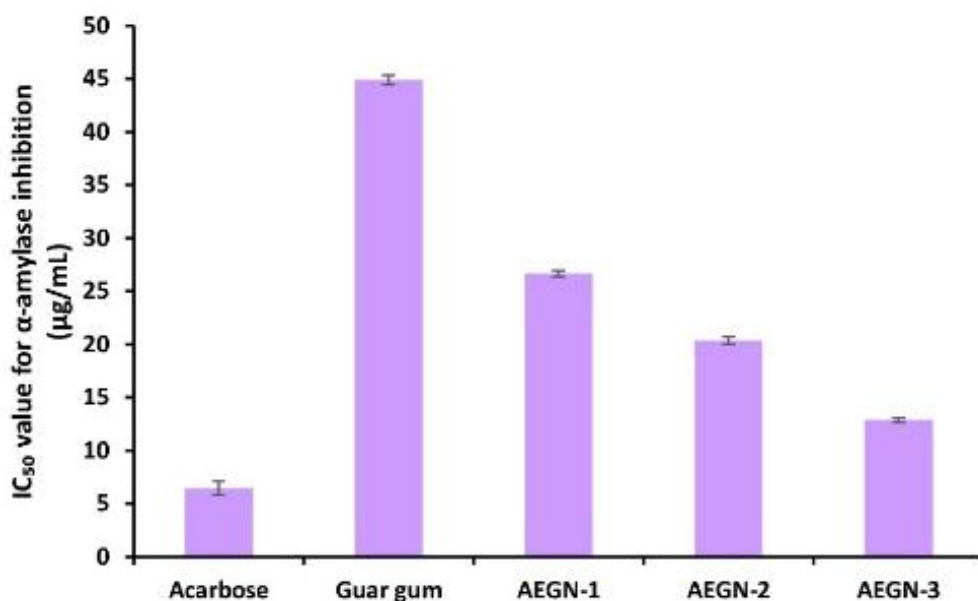


Figure 6.26: IC₅₀ values for α-amylase inhibitory effects of acarbose, guar gum, and AEGN formulations (μg/mL). Values are presented as mean ± SEM (n = 3).

6.3.6 α-glucosidase inhibitory activity of AEGN

The α-glucosidase inhibitory activity of the formulations was assessed, with acarbose serving as the reference standard. As depicted in the figure 6.27, acarbose exhibited potent α-glucosidase inhibition, with an IC₅₀ value of 5.5 ± 0.25 μg/mL. Guar gum showed moderate inhibitory activity with an IC₅₀ value of 48.62 ± 0.18 μg/mL. Among the formulations, AEGN-3 demonstrated the most significant α-glucosidase inhibition, with an IC₅₀ value of 6.97 ± 0.21 μg/mL, followed by AEGN-2 at 16.48 ± 0.20 μg/mL and AEGN-1 at 25.28 ± 0.15 μg/mL. These findings indicate that the formulations, especially AEGN-3, possess promising α-glucosidase inhibitory activity and could be explored further as potential anti-diabetic treatments.

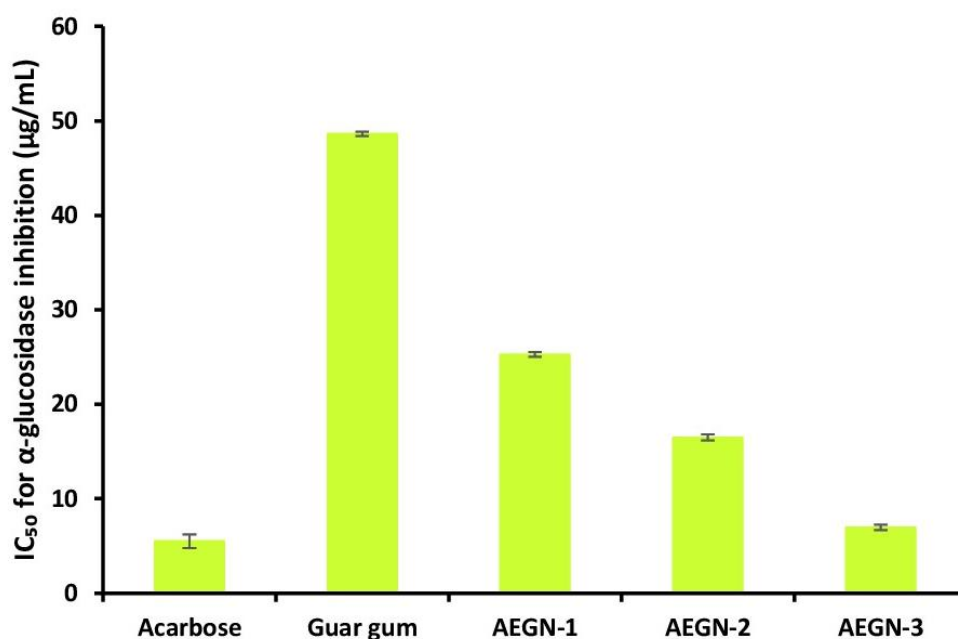


Figure 6.27: IC₅₀ values for α-glucosidase inhibitory effects of acarbose, guar gum, and AEGN formulations (μg/mL). Values are presented as mean ± SEM (n = 3).

6.3.7 AEGN nanoformulations exhibit excellent biocompatibility in MTT assay

Preclinical cytotoxicity evaluation was performed to ensure the safety and biocompatibility of AEGN formulations before *in vivo* anti-diabetic studies. The cytotoxic effects of AEGN-1, AEGN-2, and AEGN-3 were assessed on normal cell lines (BHK-21, L929, L6, and 3T3) as well as metabolically relevant cells (Caco-2 and HepG2).

As shown in Figure 6.28, all three formulations maintained high cell viability (>60%) across all tested concentrations up to 100 μg/mL, indicating minimal cytotoxicity. In BHK-21 cells, the IC₅₀ values were 142.91 ± 0.13 μg/mL (AEGN-1), 153.99 ± 0.14 μg/mL (AEGN-2), and 181.44 ± 0.24 μg/mL (AEGN-3), while acarbose and guar gum showed IC₅₀ values of 129.59 ± 0.14 μg/mL and 198.17 ± 0.34 μg/mL, respectively. Similarly, in L929 fibroblast cells, IC₅₀ values were 215.17 ± 0.29 μg/mL (AEGN-1), 245.37 ± 0.36 μg/mL (AEGN-2), and 317.28 ± 0.29 μg/mL (AEGN-3), compared to 141.81 ± 0.38 μg/mL and 298.74 ± 0.23 μg/mL for acarbose and guar gum. In L6 cells, the formulations preserved viability above 60% at 100 μg/mL, with IC₅₀ values of 159.62 ± 0.41 μg/mL (AEGN-1), 161.75 ± 0.35 μg/mL (AEGN-2), and 171.10 ± 0.31 μg/mL (AEGN-3). In 3T3 cells, IC₅₀ values were 197.20 ± 0.17 μg/mL (AEGN-1), 215.60 ± 0.15 μg/mL (AEGN-2), and 227.96 ± 0.22 μg/mL (AEGN-3), while acarbose and guar gum showed 185.48 ± 0.33 μg/mL and 260.27 ± 0.14 μg/mL, respectively.

In metabolically relevant Caco-2 and HepG2 cells, the formulations demonstrated good biocompatibility at lower concentrations, although some reduction in cell viability was observed at higher concentrations. In Caco-2 cells, IC_{50} values were $154.73 \pm 0.19 \mu\text{g/mL}$ (AEGN-1), $158.47 \pm 0.16 \mu\text{g/mL}$ (AEGN-2), and $285.29 \pm 0.19 \mu\text{g/mL}$ (AEGN-3), with acarbose and guar gum showing $151.35 \pm 0.07 \mu\text{g/mL}$ and $195.03 \pm 0.62 \mu\text{g/mL}$, respectively. In HepG2 cells, IC_{50} values were $196.47 \pm 0.17 \mu\text{g/mL}$ (AEGN-1), $196.34 \pm 0.17 \mu\text{g/mL}$ (AEGN-2), and $247.52 \pm 0.11 \mu\text{g/mL}$ (AEGN-3), compared to $198.41 \pm 0.11 \mu\text{g/mL}$ and $233.16 \pm 0.14 \mu\text{g/mL}$ for acarbose and guar gum. Collectively, these results indicate that the AEGN formulations are highly biocompatible with normal and metabolically relevant cells, supporting their safe use in further *in vivo* anti-diabetic evaluations. The slight reduction in viability at higher concentrations emphasizes the importance of dose optimization to ensure safety in target tissues.

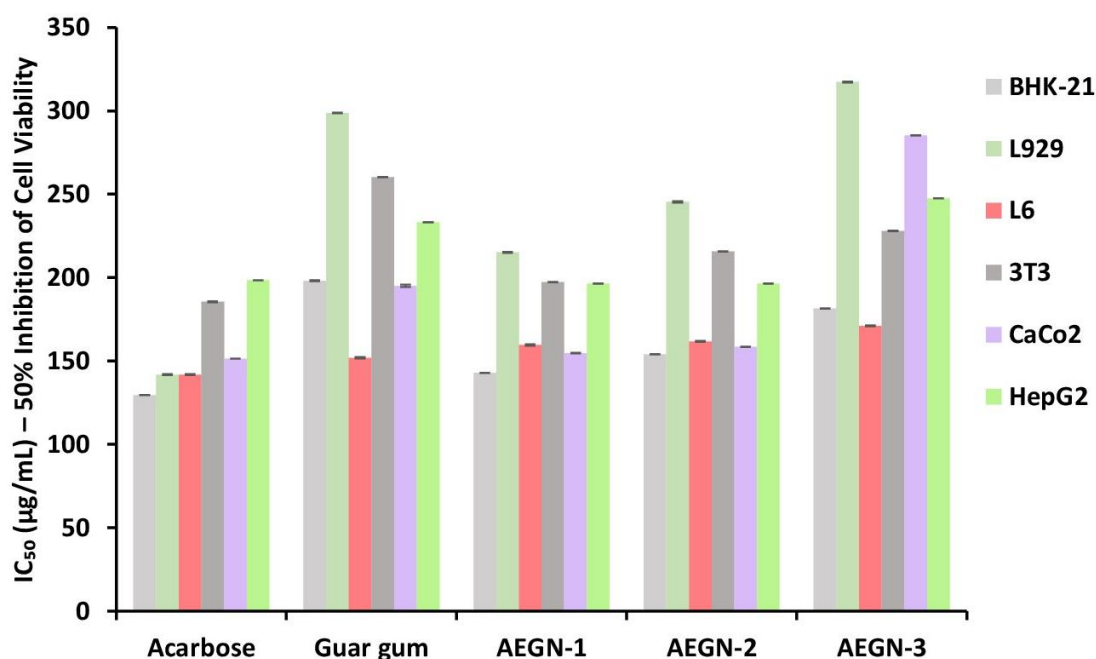


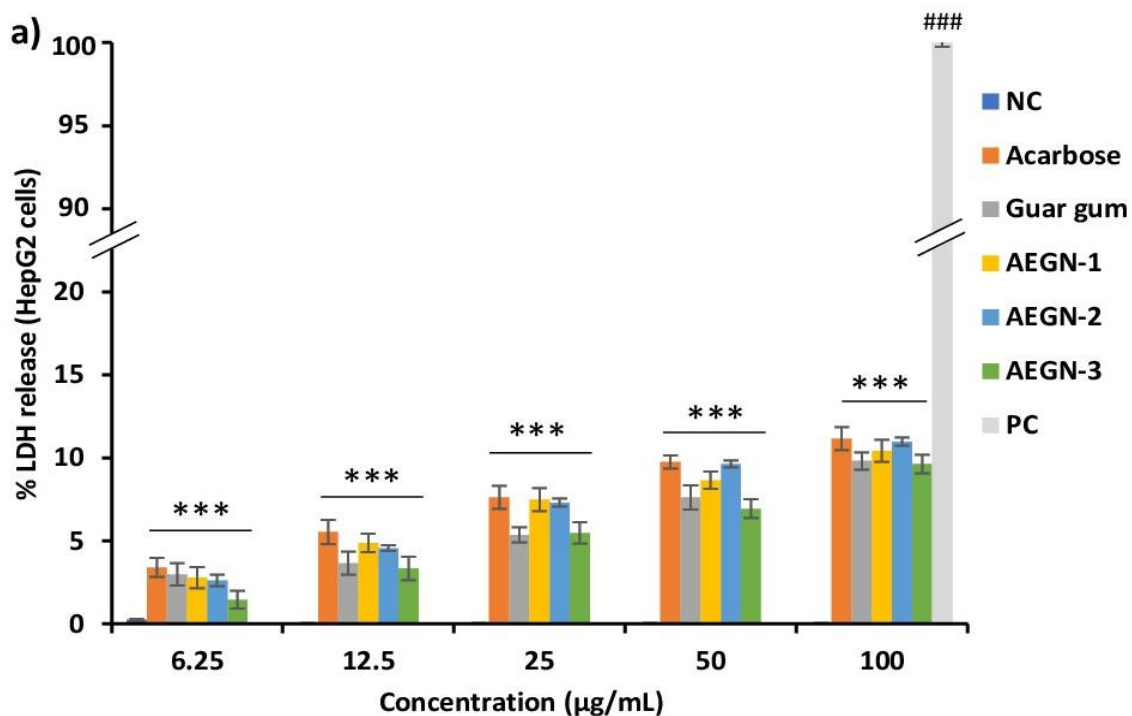
Figure 6.28: IC_{50} values for cell viability of acarbose, guar gum, and AEGN formulations ($\mu\text{g/mL}$) on BHK-21, L929, L6, 3T3, Caco-2, and HepG2 cells. Values are presented as mean $\pm$ SEM ($n = 3$).

6.3.8 AEGN Nanoformulations cause minimal membrane damage in LDH assay

The assessment of LDH release in HepG2 and L6 cells revealed distinct concentration-dependent cytotoxicity profiles for acarbose, guar gum, and the AEGN formulations. As depicted in Figure 6.29a, acarbose exhibited a gradual increase in LDH release in HepG2 cells, starting from 3.41% at $6.25 \mu\text{g/mL}$ to 11.17% at $100 \mu\text{g/mL}$, indicating dose-dependent

cytotoxicity. Guar gum demonstrated slightly lower LDH levels, ranging from 2.99% to 9.82%, reflecting better biocompatibility compared to acarbose. Among the AEGN formulations, AEGN-3 showed the lowest LDH release across all concentrations, from 1.46% at 6.25 $\mu\text{g/mL}$ to 9.64% at 100 $\mu\text{g/mL}$, signifying superior membrane stability and reduced cytotoxicity. AEGN-1 and AEGN-2 displayed moderate LDH release, with AEGN-2 reaching 10.98% at 100 $\mu\text{g/mL}$, slightly higher than AEGN-1 at 10.43%. These findings underscore the enhanced biocompatibility of AEGN-3 in HepG2 cells, likely due to its optimized formulation.

In L6 cells, acarbose induced a marked increase in LDH release, starting from 4.38% at 6.25 $\mu\text{g/mL}$ and escalating to 19.01% at 100 $\mu\text{g/mL}$, indicating substantial cytotoxic stress. Guar gum showed a more favorable profile, rising from 2.71% to 11.29%, suggesting better cellular tolerance. Notably, AEGN-2 exhibited a controlled LDH release pattern, ranging from 2.59% to 12.71%, highlighting its relatively safer profile in L6 cells. AEGN-1 followed a similar trajectory, reaching 13.95% at the highest concentration, while AEGN-3 displayed moderate LDH release from 3.82% to 13.39%, which, although higher than in HepG2 cells, still indicates reduced cytotoxicity compared to acarbose. Collectively, these results demonstrate that AEGN formulations, particularly AEGN-3, exhibit superior cytocompatibility compared to acarbose, with guar gum also providing a protective effect, especially in L6 cells (Figure 6.29b). This highlights the potential of AEGN-3 as a safer therapeutic candidate with reduced membrane damage across different cellular environments.



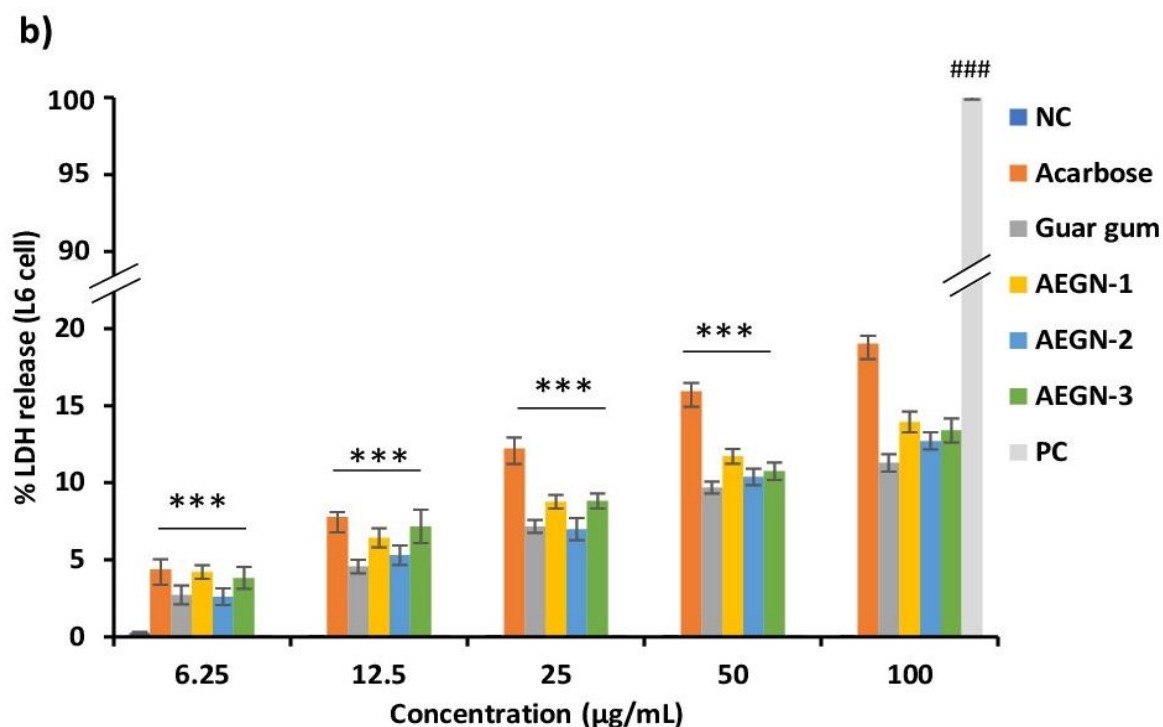


Figure 6.29: Percentage of LDH release in (a) HepG2 and (b) L6 cells after treatment with acarbose, guar gum, and AEGN formulations at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL. Values are presented as mean ± SEM (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ###p < 0.001 compared to normal control (NC), and ***p < 0.001 compared to positive control (PC).

6.3.9 Hemolysis assay reveals safe interaction of AEGNs with Red Blood Cells

The hemotoxicity assay was performed to evaluate the biocompatibility of the nanoformulations with blood components. The assay included varying concentrations (1, 2, 5, and 10 µg/mL) of acarbose, guar gum, AEGN-1, AEGN-2, and AEGN-3. Hemolysis values below 10% are categorized as non-hemolytic, whereas values exceeding 25% are indicative of hemolytic potential. Triton X-100, serving as the positive control, induced complete hemolysis (100%), while PBS as the negative control showed minimal hemolysis (Figure 6.30).

At the highest concentration (10 µg/mL), acarbose exhibited $2.85 \pm 0.04\%$ hemolysis, while the inclusion of guar gum at the same concentration significantly reduced hemolysis to $0.88 \pm 0.04\%$. The combination of acarbose with guar gum also demonstrated a reduction in hemolysis at 10 µg/mL compared to acarbose alone. Notably, AEGN-1, AEGN-2, and AEGN-3 exhibited hemolysis rates of $1.78 \pm 0.04\%$, $1.5 \pm 0.03\%$, and $1.3 \pm 0.05\%$, respectively ($p < 0.001$),

confirming their non-hemolytic nature. These findings suggest that the developed AEGNs are hemocompatible, highlighting their potential safety for therapeutic application.

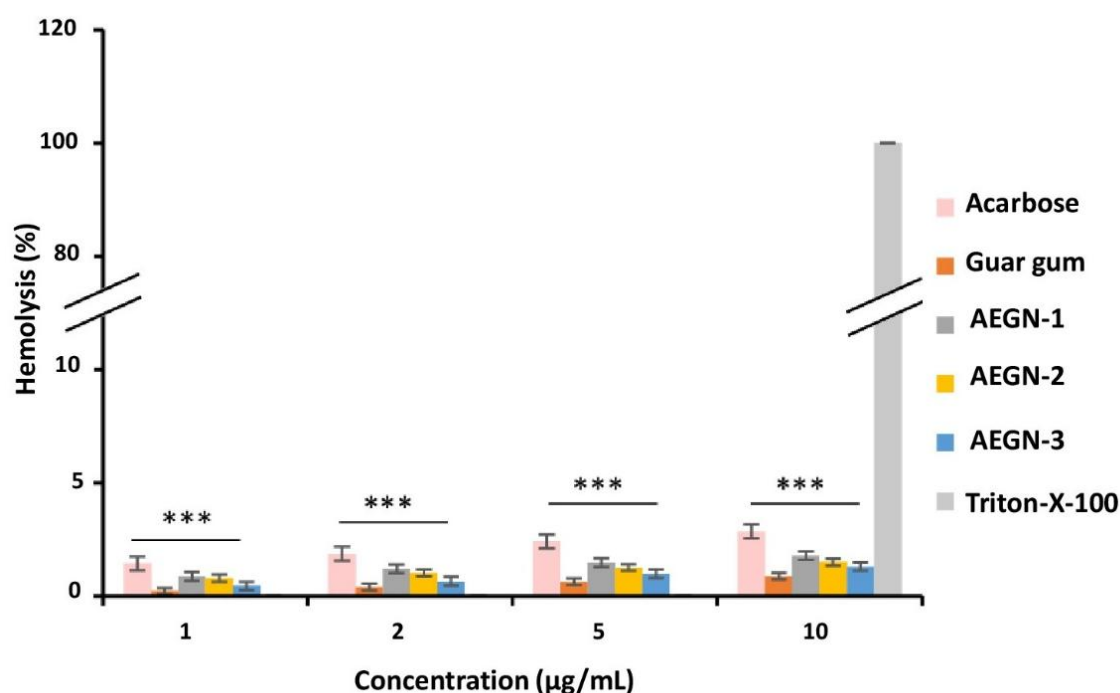


Figure 6.30: Percentage of hemolysis after treatment with acarbose, guar gum, and AEGN formulations at concentrations of 1, 2, 5, and 10 µg/mL. Values are presented as mean ± SEM (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison post hoc test. Statistical significance is indicated as ***p < 0.001 compared to Triton X-100 (positive control).

6.3.10 AEGN formulations mitigate lipid peroxidative damage in cells exposed to high glucose

LPO levels were evaluated in four different cell lines including BHK-21, L6, HepG2 and 3T3 under both low glucose and high glucose conditions to assess oxidative stress induced by hyperglycemia. As shown in the figure 6.31a, exposure to high glucose resulted in a significant increase in LPO levels across all cell lines (p < 0.001), indicating oxidative damage, with fold increases of 4.16-fold in 3T3 cells, 7.95 in BHK-21, and 2.26 in HepG2 cells, and 9.13 in L6 cells. Compared to the low glucose control, all treatment groups significantly reduced LPO levels in 3T3 cells (p < 0.01) and HepG2 cells (p < 0.001) under low-glucose conditions. However, no statistically significant reductions were observed in BHK-21 and L6 cells, although the treatments were still effective in reducing LPO levels in these cell lines.

Treatment with Acarbose, Guar gum and AEGN formulations under high glucose conditions led to a dose dependent reduction in LPO levels in all cell lines. Compared to high glucose control, acarbose at 50 µg/mL and 100 µg/mL reduced LPO levels ($p < 0.001$), by 1.15-fold and 1.33-fold in 3T3 cells; 1.65-fold and 2.06-fold in BHK-21 cells; 1.13-fold and 1.39-fold in HepG2 cells; and 1.02-fold and 1.22-fold in L6 cells. In comparison, Guar gum exhibited superior antioxidant activity ($p < 0.001$), with reductions of 1.25-fold and 1.51-fold in 3T3 cells; 1.39-fold and 1.65-fold in BHK-21 cells; 1.44-fold and 1.77-fold in HepG2 cells; and 1.36-fold and 1.59-fold in L6 cells.

The huge reduction in LPO levels were observed when all cells were treated with formulations, when compared to acarbose and guar gum treatments. AEGN-1 at 50 µg/mL and 100 µg/mL led to reductions ($p < 0.001$) by 1.41-fold and 1.69-fold in 3T3 cells; 1.70-fold and 2.04-fold in BHK-21 cells; 1.61-fold and 1.92-fold in HepG2 cells; and 1.76-fold and 2.30-fold in L6 cells. AEGN-2 showed relatively greater reductions than AEGN-1 in all cases ($p < 0.001$). Notably, AEGN-3 demonstrated the most pronounced antioxidant effect, particularly at 100 µg/mL ($p < 0.001$), with LPO levels reduced by 3.55-fold in 3T3 cells, 5.73-fold in BHK-21, 3.43-fold in HepG2, and 7.90-fold in L6 cells. These findings underscore the superior efficacy of AEGN formulations, especially AEGN-3, in mitigating high glucose-induced oxidative stress compared to acarbose and guar gum. Overall, the results confirm that high glucose triggers oxidative stress by increasing LPO. Among all treatments, AEGN-3 at higher concentration was the most effective in reducing oxidative damage and shows strong potential to protect cells from glucose induced stress.

6.3.11 Effect of AEGN formulations on SOD levels in high glucose exposed cells

Figure 6.31b illustrates the impact of various treatments in alleviating high glucose-induced oxidative stress by enhancing SOD levels in BHK-21, L6, HepG2, and 3T3 cells under both low glucose and high glucose conditions. Although these enhancements were not statistically significant under low glucose conditions, a notable exception was observed in HepG2 cells, which exhibited a significant increase in SOD levels following AEGN-3 treatment at 50 and 100 µg/mL ($p < 0.01$ and $p < 0.001$, respectively). Nevertheless, the treatments demonstrated greater efficacy in mitigating oxidative stress. Compared to low glucose controls, high glucose exposure significantly reduced SOD levels across all cell lines ($p < 0.001$). The reductions were 8.5-fold in 3T3 cells, 6.29-fold in BHK-21 cells, 2.29-fold in HepG2 cells, and 6.37-fold in L6 cells.

Treatment with Acarbose, Guar gum and AEGN formulations under high glucose conditions led to a dose dependent increase in SOD levels in all cell lines. Specifically, Acarbose at 50 µg/mL and 100 µg/mL dose were effective in increasing SOD levels, by 1.44-fold and 1.76-fold in 3T3 cells ($p < 0.001$), 2.92-fold and 3.17-fold ($p < 0.001$) in BHK-21 cells, 1.29-fold and 1.34-fold ($p < 0.001$), and 3.55-fold and 4.03-fold in L6 cells ($p < 0.001$), respectively. Guar gum treatment at both doses significantly enhanced SOD levels by 2.10-fold and 2.49-fold in 3T3 cells ($p < 0.001$), 2.85-fold and 3.86-fold in BHK-21 cells ($p < 0.001$), 1.30-fold and 1.75-fold in HepG2 cells ($p < 0.001$), and 3.98-fold and 4.20-fold in L6 cells ($p < 0.001$). Both acarbose and guar gum, at their respective doses, showed improvement in oxidative stress by increasing SOD levels when compared to the high glucose control group.

In contrast, all AEGN treatments resulted in a significant increase in SOD levels across all cell lines ($p < 0.001$). For instance, AEGN-1 increased the SOD levels by 2.46-fold and 2.71-fold in 3T3 cells, followed by 3.02-fold and 3.97-fold in BHK-21 cells ($p < 0.001$), 1.41-fold and 1.81-fold ($p < 0.001$) in HepG2 cells, and 3.55-fold, 3.63-fold in L6 cells ($p < 0.001$). SOD levels were also significantly increased ($p < 0.001$) when cells were treated with AEGN-2, compared to AEGN-1. However, among AEGN-1 and AEGN-2, notable improvements in SOD levels were observed with AEGN-3 treatment in all cells at both doses ($p < 0.001$). Specifically, AEGN-3 treatment led to a 3.57-fold and 3.85-fold increase in SOD levels in 3T3 cells, 5.80-fold in BHK-21 cells, 2.33-fold and 2.59-fold in HepG2 cells, and 5.37-fold, 6.06-fold in L6 cells, indicating reduction in HG-induced oxidative stress through the enhancement of SOD levels.

6.3.12 Restoration of catalase activity by AEGN formulations under hyperglycemic conditions

To assess the antioxidant potential of AEGN formulations, catalase activity was evaluated in cells maintained under low and high glucose conditions. The results showed that all treatment groups appeared to maintain catalase levels across all cell lines compared to the low glucose control. However, under low glucose conditions, treatments in 3T3, BHK-21, and L6 cells did not reach any statistical significance, although catalase activity was effectively maintained. In contrast, under low glucose conditions, treatment with AEGN-2 at both doses significantly increased catalase activity in HepG2 cells ($p < 0.05$). Additionally, AEGN-3 at 100 µg/mL led to a significant enhancement in catalase activity ($p < 0.01$), as shown in the Figure 6.31c.

Under high glucose conditions, a notable enhancement in catalase activity was observed across all cell lines following different treatments. For instance, treatment with acarbose at 50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ led to 1.79-fold and 2.33-fold increases in 3T3 cells ($p < 0.001$), 2.26-fold and 2.62-fold increases in BHK-21 cells ($p < 0.001$), 2.16-fold and 2.99-fold increases in HepG2 cells ($p < 0.001$), and 1.28-fold and 2.55-fold increases in L6 cells ($p < 0.001$). Treatment with guar gum at 100 $\mu\text{g/mL}$ also significantly increased catalase activity ($p < 0.001$), with 2.52-fold, 3.34-fold, 3.69-fold, and 2.16-fold increases observed in 3T3, BHK-21, HepG2, and L6 cells, respectively, indicating that both acarbose and guar gum effectively mitigate oxidative stress by restoring catalase activity under high glucose exposure.

In contrast to acarbose and guar gum treatments, all AEGN formulations demonstrated a more pronounced enhancement in catalase activity across all cell lines ($p < 0.001$). AEGN-1 at 100 $\mu\text{g/mL}$ enhanced catalase activity by 2.48-fold in 3T3 cells, 3.21-fold in BHK-21 cells, 3.37-fold in HepG2 cells, and 2.22-fold in L6 cells. This effect was even greater with AEGN-2 at both 50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ ($p < 0.001$), resulting in 2.85-fold and 3.08-fold increases in 3T3 cells, 3.12-fold and 3.80-fold increases in BHK-21 cells, 3.39-fold and 4.14-fold increases in HepG2 cells, and 2.15-fold and 2.73-fold increases in L6 cells, respectively. Among all AEGN formulations, AEGN-3 showed the most significant improvement at both doses ($p < 0.001$), leading to 3.58-fold and 4.11-fold increases in 3T3 cells, 4.19-fold and 4.60-fold increases in BHK-21 cells, 4.75-fold and 5.35-fold increases in HepG2 cells, and 3.08-fold and 3.60-fold increases in L6 cells, respectively.

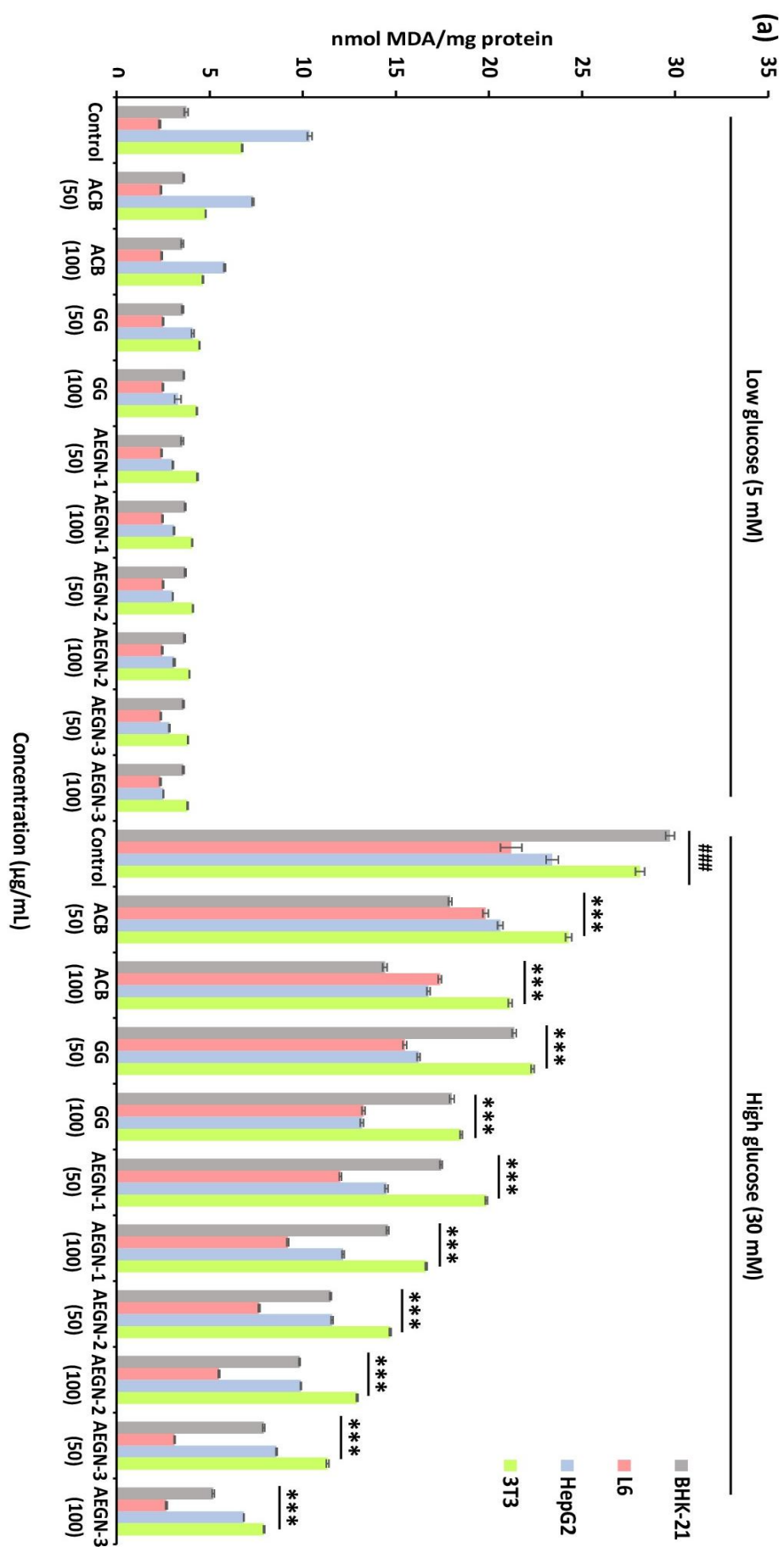
6.3.13 AEGN treatment increases GST activity under hyperglycemic conditions

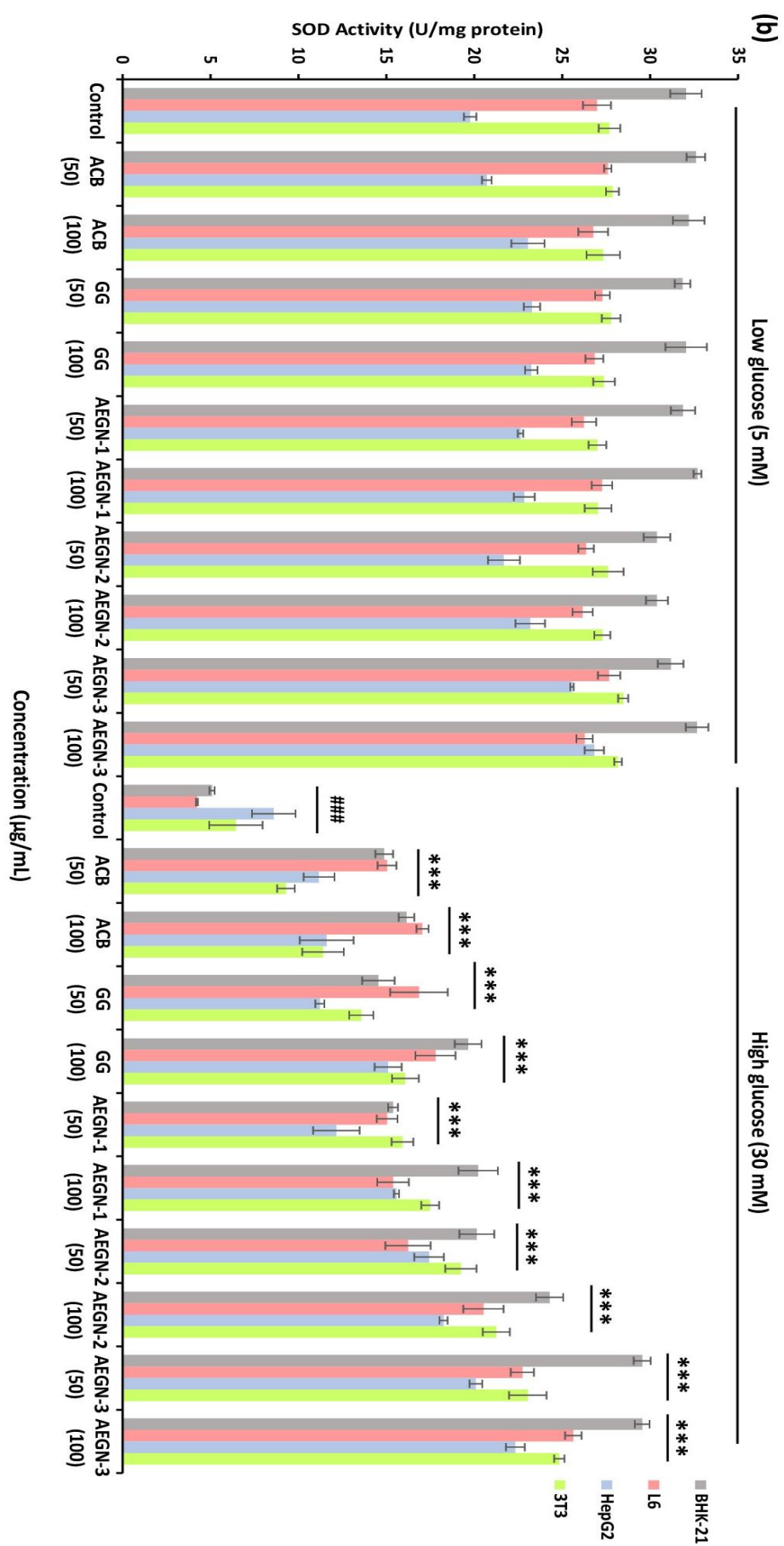
Consistent with the SOD and catalase results, cells cultured under low glucose conditions without any treatment showed an increase in GST activity. Upon receiving treatment, however, no statistical significance was observed, although all treatments effectively maintained GST activity across all cell lines, comparable to the low glucose control. In contrast, GST activity was significantly reduced in cells cultured under high glucose conditions without treatment. Specifically, this reduction was observed as 2.89-fold in 3T3 cells, 3.98-fold in BHK-21 cells, 5.30-fold in HepG2 cells, and 5.22-fold in L6 cells, indicating that exposure to high glucose conditions exacerbates oxidative stress.

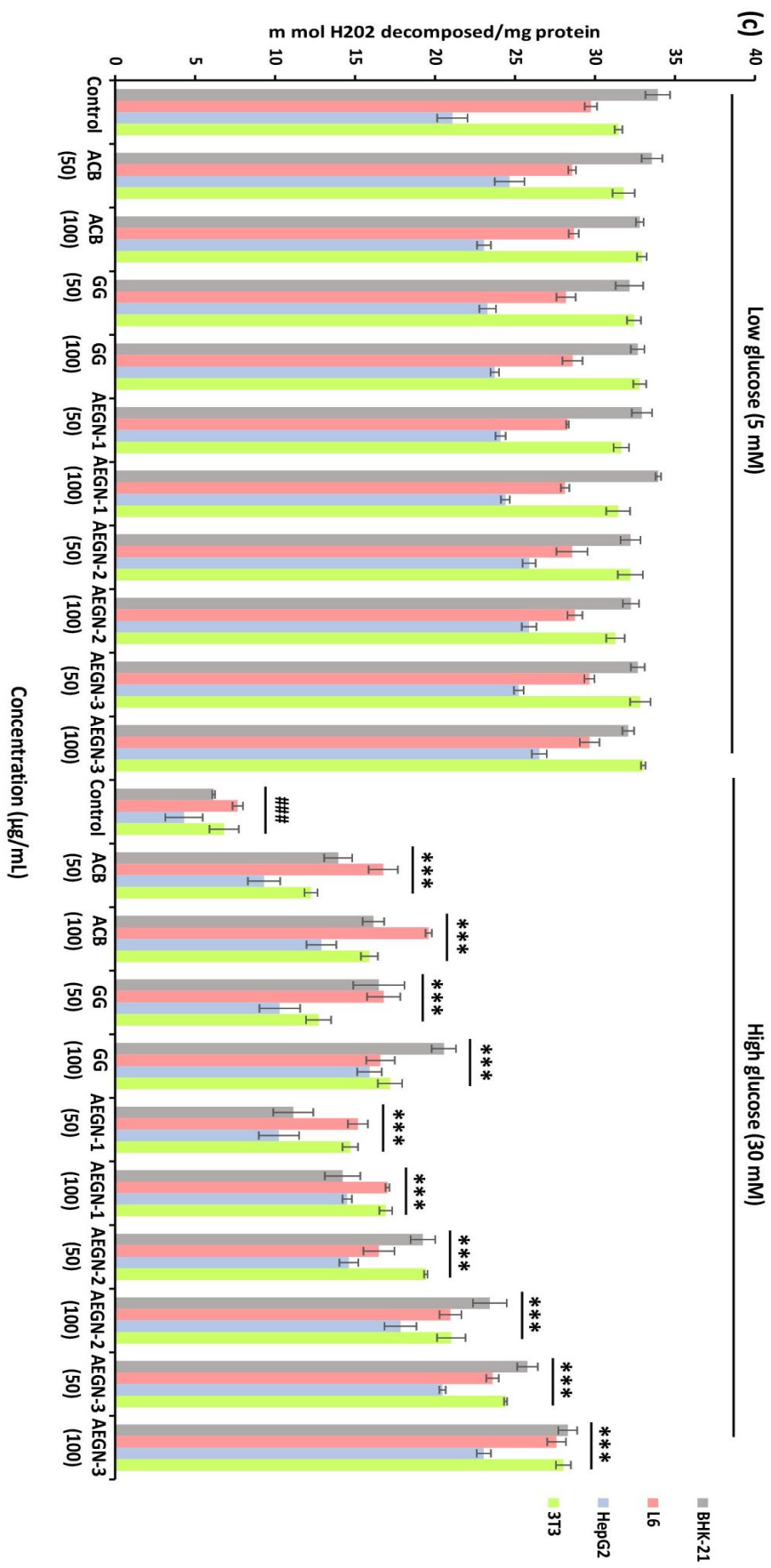
However, this reduction in GST activity was improved in a dose-dependent manner when high glucose-exposed cells were treated with acarbose and guar gum. For example, acarbose treatment at 50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ significantly increased GST activity ($p < 0.001$), leading

to 1.33-fold and 1.45-fold increases in 3T3 cells, 1.56-fold and 1.92-fold increases in BHK-21 cells, 2.39-fold and 2.69-fold increases in HepG2 cells, and 3.08-fold and 3.47-fold increases in L6 cells, respectively. Similarly, guar gum at the same doses significantly elevated GST activity ($p < 0.001$), showing 1.11-fold and 1.28-fold increases in 3T3 cells, 1.85-fold and 2.13-fold in BHK-21 cells, 2.78-fold and 3.32-fold in HepG2 cells, and 2.80-fold and 3.38-fold in L6 cells. More notably, high glucose-exposed cells treated with AEGN formulations showed superior restoration of GST activity. Treatment with AEGN-1 at 100 $\mu\text{g/mL}$ significantly improved GST activity ($p < 0.001$), resulting in 1.56-fold increase in 3T3 cells, 2.30-fold in BHK-21 cells, 3.46-fold in HepG2 cells, and 3.20-fold in L6 cells. AEGN-2 at the same dose further enhanced GST activity ($p < 0.001$), with 1.61-fold in 3T3 cells, 2.67-fold in BHK-21 cells, 4.64-fold in HepG2 cells, and 3.43-fold in L6 cells.

Importantly, AEGN-3 demonstrated the highest antioxidant potential, with both 50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ treatments significantly improving GST activity ($p < 0.001$) beyond that achieved with AEGN-1 and AEGN-2. Specifically, AEGN-3 treatment resulted in 1.82-fold and 2.37-fold increases in 3T3 cells, 3.20-fold and 3.55-fold in BHK-21 cells, 4.69-fold and 5.10-fold in HepG2 cells, and 4.27-fold and 4.86-fold in L6 cells, respectively, as depicted in Figure 6.31d. These findings suggest that AEGN-3, even at lower doses, exerts a stronger antioxidant effect in mitigating HG-induced oxidative stress compared to other treatments.







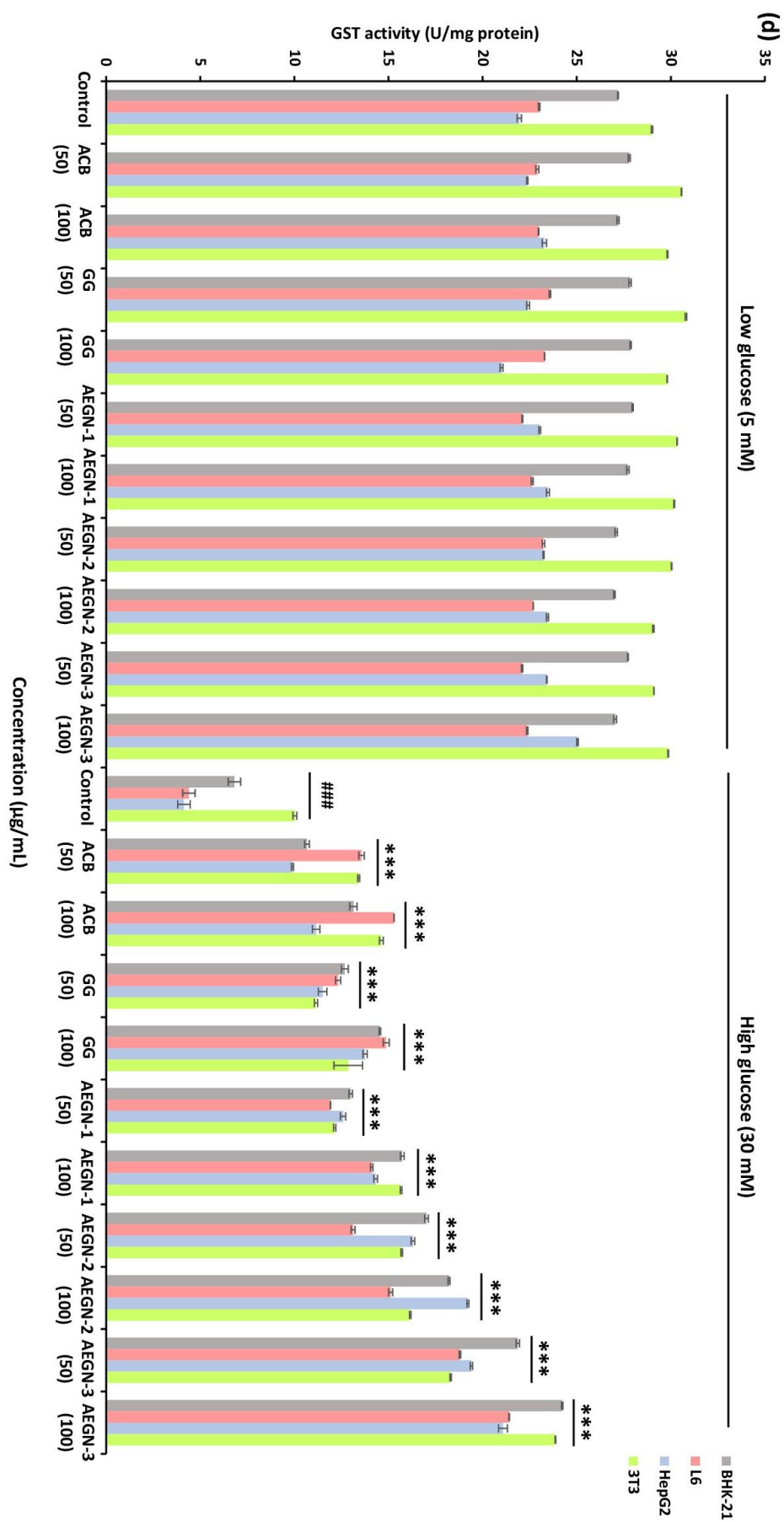


Figure 6.31: Effect of acarbose (ACB), guar gum (GG), and AEGNs on oxidative stress and antioxidant enzyme activities in BHK-21, L6, HepG2, and 3T3 cell lines under low (5 mM) and high (30 mM) glucose conditions at concentrations of 50 and 100 µg/mL. **(a)** Lipid peroxidation (LPO) levels assessed by measuring malondialdehyde (MDA) content (nmol MDA/mg protein); **(b)** Superoxide dismutase (SOD) activity expressed as U/mg protein; **(c)** Catalase activity expressed as mmol H₂O₂ decomposed per mg protein; **(d)** Glutathione S-transferase (GST) activity expressed as U/mg protein. Data are presented as mean ± SEM (n = 3). Statistical analysis was performed using two-way analysis of variance (ANOVA) to evaluate the effects of glucose condition and treatment, followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ####p < 0.001 compared to the corresponding low glucose (5 mM) control; ***p < 0.001 compared to the high glucose (30 mM) control.

6.4 *In vivo* pharmacological effects of AEGN-3 in HFD-fed, STZ-induced diabetic rats

6.4.1 Administration of AEGN-3 mitigates body weight gain in diabetic rats

As shown in Figure 6.32, HFD-fed, STZ-induced diabetic rats exhibited a significant 1.2-fold increase in body weight compared to the normal control group ($p < 0.001$), confirming successful establishment of the diabetic model. Therapeutic intervention mitigated this increase, with acarbose treatment resulting in a 1.08-fold reduction in body weight relative to the diabetic control group, while guar gum also produced a 1.08-fold reduction ($p < 0.05$). Administration of AEGN-3 demonstrated a more pronounced weight-modulatory effect, inducing 1.12-fold and 1.15-fold reductions in body weight at doses of 25 mg/kg and 50 mg/kg, respectively, indicating a dose-dependent improvement.

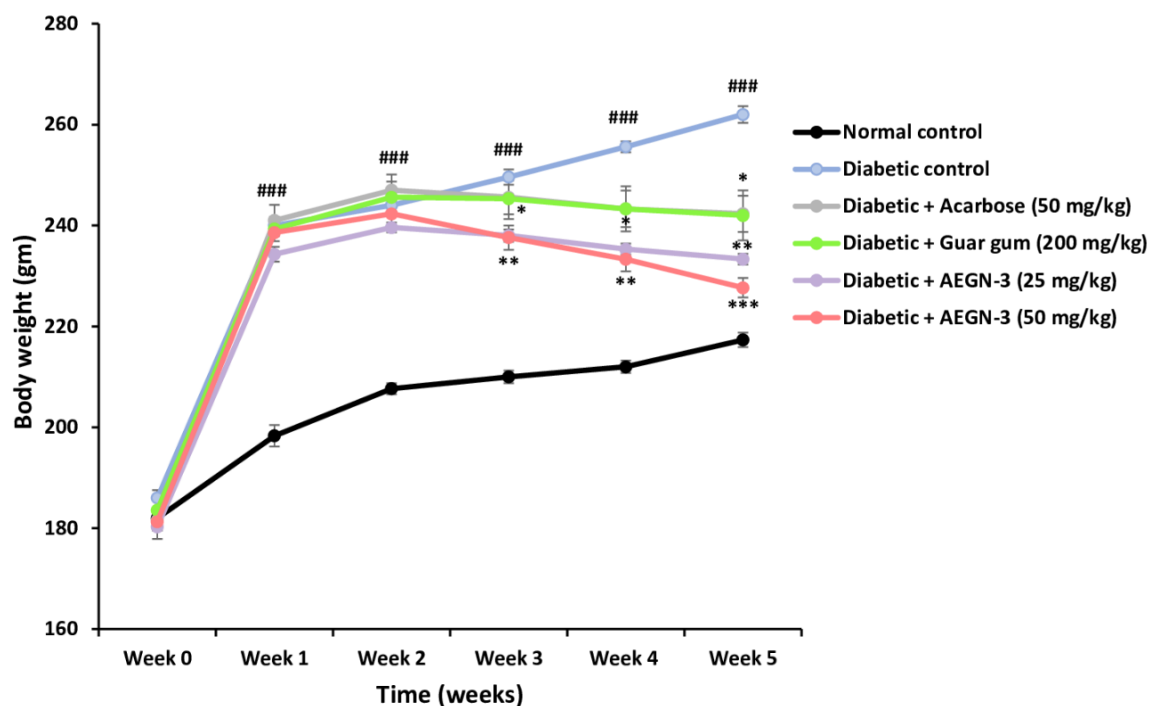


Figure 6.32: Effect of acarbose (50 mg/kg), guar gum (200 mg/kg), and AEGN-3 (25 and 50 mg/kg) on body weight in diabetic rats over 5 weeks. Data are mean $\pm$ SEM (n = 6). Statistical analysis was performed using two-way repeated measures ANOVA followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ###p < 0.001 compared to normal control; *p < 0.05, **p < 0.01, ***p < 0.001 compared to diabetic control.

6.4.2 Administration of AEGN-3 alleviates blood glucose levels in diabetic rats

As expected, HFD-fed, STZ-induced diabetic rats exhibited a significant 3.57-fold elevation in blood glucose levels compared to the normal control group ($p < 0.001$), confirming successful establishment of the diabetic model. Therapeutic intervention significantly reduced hyperglycemia, with acarbose treatment producing a 2.06-fold decrease in blood glucose levels relative to the diabetic control group ($p < 0.001$), while guar gum resulted in a 1.65-fold reduction ($p < 0.05$). Notably, AEGN-3 demonstrated a more pronounced antihyperglycemic effect, reducing blood glucose levels by 2.77-fold at 25 mg/kg ($p < 0.01$) and 3.37-fold at 50 mg/kg ($p < 0.001$), indicating a dose-dependent improvement in glycemic control (Figure 6.33).

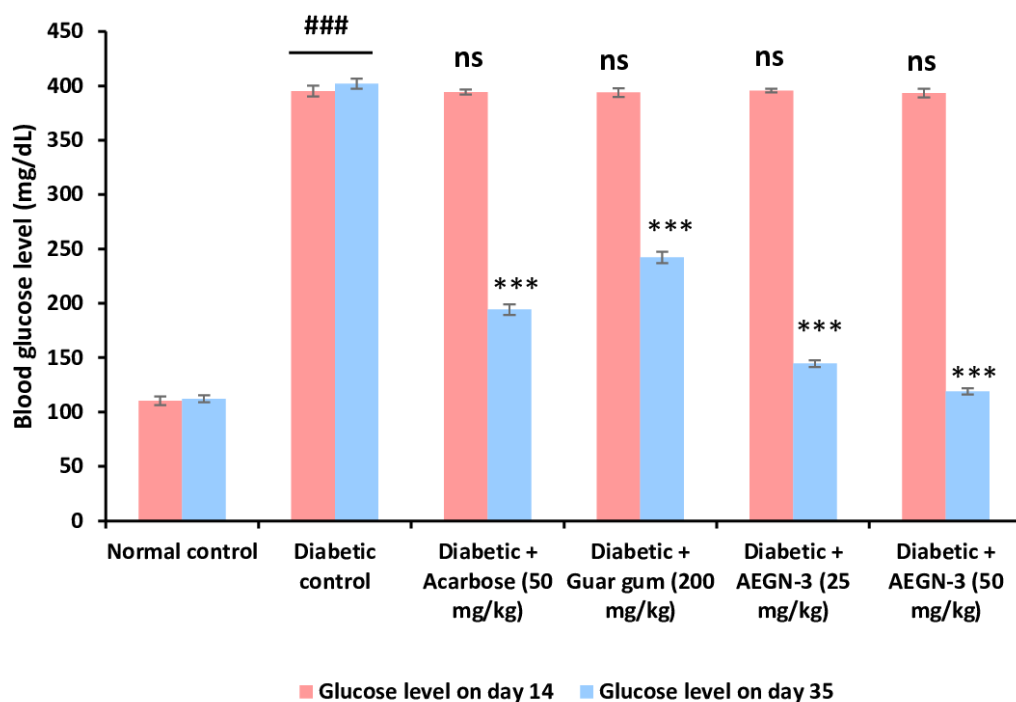


Figure 6.33: Effect of acarbose (50 mg/kg), guar gum (200 mg/kg), and AEGN-3 (25 and 50 mg/kg) on blood glucose levels (mg/dL) in diabetic rats on days 14 and 35. Data are presented as mean $\pm$ SEM (n = 6). Statistical analysis was performed using two-way repeated measures ANOVA followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ###p < 0.001 compared to normal control; ***p < 0.001 compared to day 14; ns = not significant.

6.4.3 Administration of AEGN-3 reduces diarrhoeal frequency in diabetic rats

The diarrhoea scores from day 1 to day 35 revealed distinct differences in fecal consistency across the treatment groups (Table 6.2). Diarrhoea was scored based on fecal characteristics using the following criteria: 0 for normal feces or absence of feces, 1 for well-shaped wet feces, 2 for shapeless feces, and 3 for unshaped feces with large amounts of liquid. In both, normal and diabetic control groups, the scores remained consistently at 0, indicating normal feces or absence of feces throughout the study period, suggesting no adverse effects on fecal consistency. In the group treated with Acarbose (50 mg/kg), diarrhoea scores initially remained at 0 but gradually increased, reaching a score of 2 on days 17 and 18, and peaking at 3 on day 19. This reflected the development of unshaped feces with a large amount of liquid, indicative of severe diarrhoea. The score then decreased to 1-2 between days 20 to 35, showing mild diarrhoea with well-shaped wet feces or shapeless feces.

In contrast, the guar gum (200 mg/kg) group exhibited minimal changes, with the diarrhoea score remaining at 0 for most of the study, except for a brief increase to 1 on day 20, indicating well-shaped wet feces. This suggests that guar gum had a very mild effect on fecal consistency, with no prolonged diarrhoea. The group treated with AEGN-3 at 25 mg/kg showed a mild increase in the score from 0 on day 15 to a maximum of 2 on day 19, indicating shapeless feces, before returning to 0 by day 20. Similarly, the AEGN-3 at 50 mg/kg displayed a slight increase in diarrhoea scores, peaking at 1 on day 18, reflecting well-shaped wet feces, but then returning to 0 by day 19 and remaining stable throughout the study. These results suggest that while Acarbose caused significant diarrhoea, particularly around day 19, both guar gum and AEGN-3 had relatively mild and transient effects on fecal consistency.

Table 6.2: Effects of various treatments on diarrhoea in experimental rats over 35 days.

Days	Normal control	Diabetic control	Diabetic + Acarbose (50 mg/kg)	Diabetic + Guar gum (200 mg/kg)	Diabetic + AEGN-3 (25 mg/kg)	Diabetic + AEGN-3 (50 mg/kg)
1 - 14	0	0	0	0	0	0
15	0	0	0	0	0	0
16	0	0	0	0	0	0
17	0	0	2	0	0	0
18	0	0	2	0	0	1
19	0	0	3	0	0	0
20	0	0	2	0	1	0
21	0	0	2	0	1	0
22	0	0	2	0	1	0
23	0	0	3	0	0	0
24	0	0	3	0	0	0
25	0	0	3	0	0	0
26	0	0	3	0	0	0
27	0	0	2	0	0	0
28	0	0	2	0	0	0
29	0	0	2	0	0	0
30	0	0	1	0	0	0

31	0	0	1	0	0	0
32	0	0	1	0	0	0
33	0	0	1	0	0	0
34	0	0	1	0	0	0
35	0	0	1	0	0	0

6.4.4 Treatments demonstrate absence of mortality and good tolerability

Throughout the 35-day experimental period, no mortality was observed in any of the experimental groups, including the normal control, disease-induced control, and treatment groups. This indicates that both the disease induction protocol and the administered treatments were well-tolerated by the animals. The survival rate was 100% across all groups, as illustrated in table 6.3.

Table 6.3: Survival data of experimental groups over the 35-day treatment period. All animals in each group survived throughout the study, and no mortality was observed during the treatment period. At the end of the study, all animals were humanely sacrificed for further analysis.

Groups	Treatment period (days)	Number of rats before treatment	Number of rats after treatment
Normal control	35	6	6
Diabetic control	35	8	8
Diabetic + Acarbose (50 mg/kg)	35	8	8
Diabetic + Guar gum (200 mg/kg)	35	8	8
Diabetic + AEGN-3 (25 mg/kg)	35	8	8
Diabetic + AEGN-3 (50 mg/kg)	35	8	8

6.4.5 AEGN-3 administration restores relative organ weights in diabetic rats

Figure 6.34 illustrates the relative organ weights of experimental animals following treatment. The diabetic control group exhibited a pronounced increase in liver and kidney indices ($p < 0.001$ vs normal control), indicative of diabetes-associated hepatomegaly and renal hypertrophy. In contrast, pancreatic index was markedly reduced ($p < 0.001$ vs normal control), reflecting pancreatic atrophy, likely due to β -cell loss under hyperglycaemic conditions.

Treatment with acarbose partially attenuated these alterations, demonstrating moderate improvements in liver, kidney, and pancreatic indices. Guar gum treatment produced a more consistent restorative effect, with most parameters showing significant improvement compared to diabetic controls. Notably, AEGN-3 nanoformulation demonstrated a clear dose-dependent therapeutic effect. At 25 mg/kg, significant restoration of liver, kidney, and pancreatic indices was observed ($p < 0.001$ vs diabetic control). Administration at 50 mg/kg further enhanced these effects, resulting in near-normalization of organ indices, particularly for kidney and pancreas ($p < 0.001$ vs diabetic control).

In addition, diabetic animals showed a significant increase in heart index ($p < 0.01$ vs normal control), indicative of cardiac hypertrophy. Treatment groups, especially those receiving AEGN-3, exhibited a significant reduction in heart index relative to diabetic controls, suggesting cardioprotective effects. Collectively, these findings demonstrate that AEGN-3, particularly at the higher dose, effectively mitigates diabetes-induced organ dysfunction, supporting its potential as a multi-targeted therapeutic strategy.

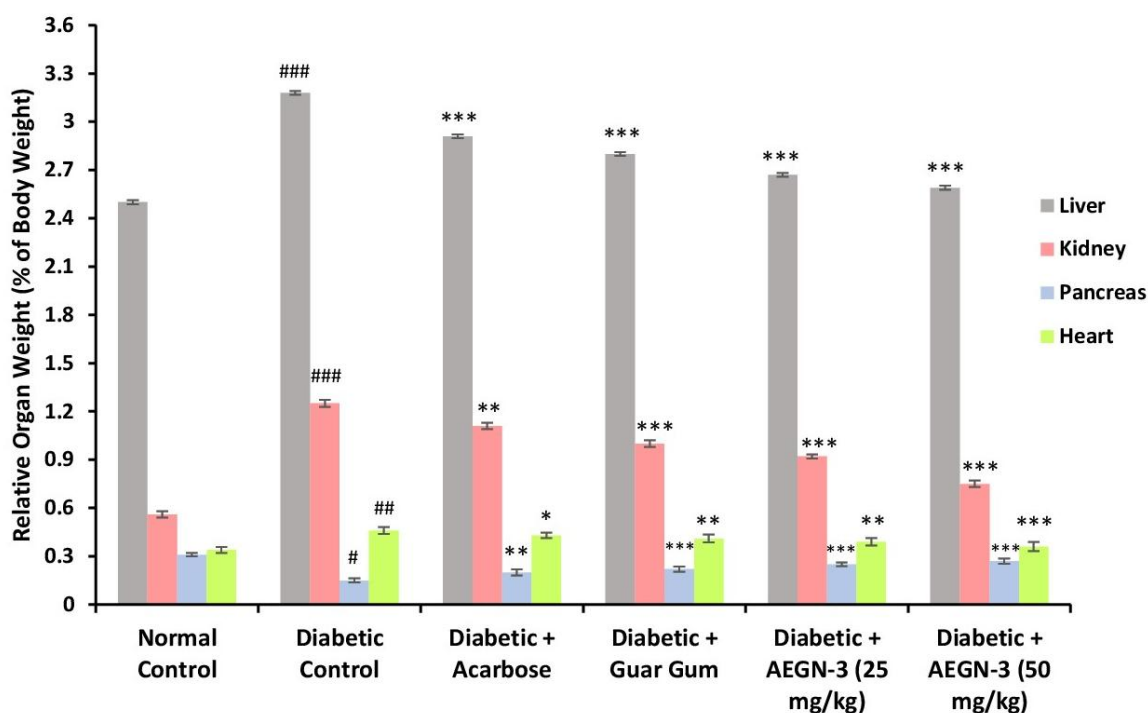


Figure 6.34: Effect of various treatments on relative organ weights in diabetic rats. Bar graph representing the relative organ weight (% of body weight) of the liver, kidney, pancreas, and heart in different experimental groups, measured at the end of the treatment period. Data are expressed as mean $\pm$ SEM (n = 6). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical analysis is indicated as ###p < 0.001 compared to normal control; *p < 0.05, **p < 0.01, ***p < 0.001 compared to diabetic control.

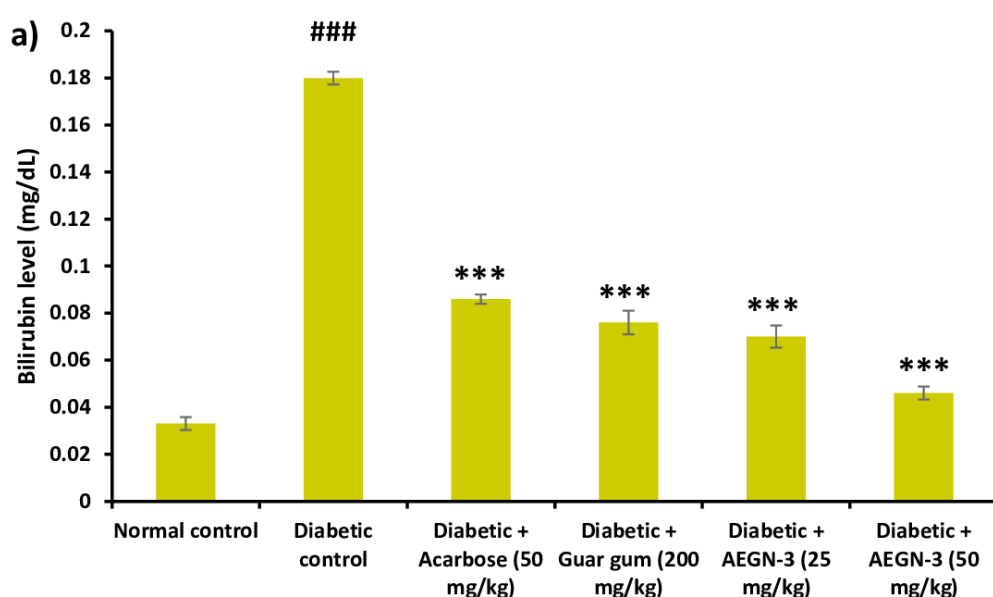
6.4.6 Administration of AEGN-3 mitigates hepatic damage by enhancing liver enzyme activity

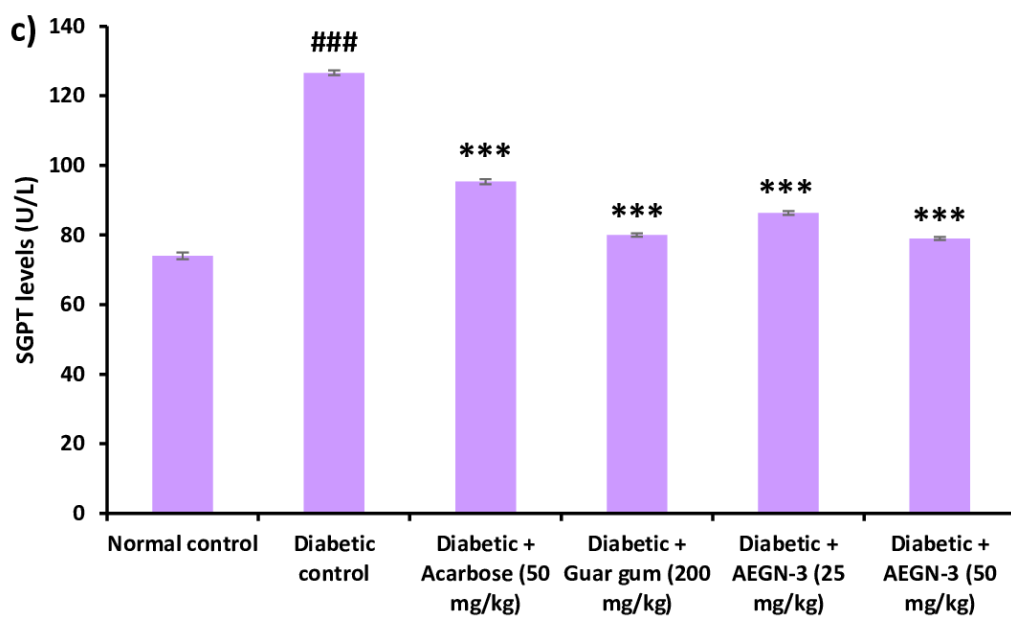
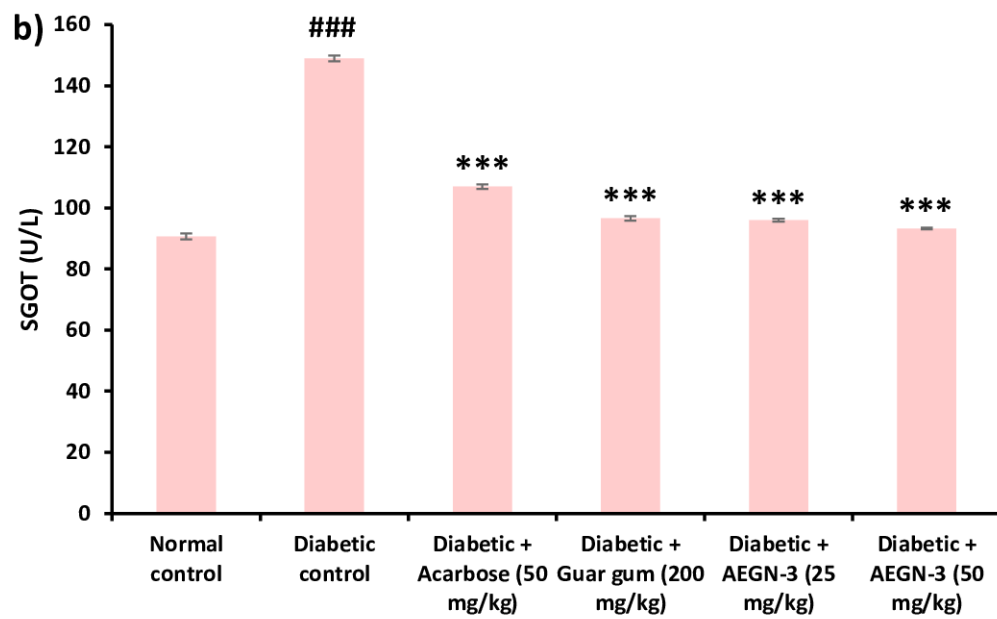
Since the liver is crucial for metabolism and detoxification, any disruption in enzymes like bilirubin, SGOT, SGPT, ALP, GGT, and LDH can cause hepatic dysfunction. To evaluate the impact of AEGN-3 therapy on hepatic enzymes, we analyzed their levels in HFD-fed, STZ-induced diabetic rats. In HFD-fed, STZ-induced diabetic rats, chronic hyperglycemia elevated bilirubin (5.45-fold; p < 0.001), SGOT (1.64-fold; p < 0.001), SGPT (1.71-fold; p < 0.001), ALP (1.63-fold; p < 0.001), GGT (3.84-fold; p < 0.001), and LDH (3.84-fold; p < 0.001), indicating significant liver damage (Figure 6.35 a-f).

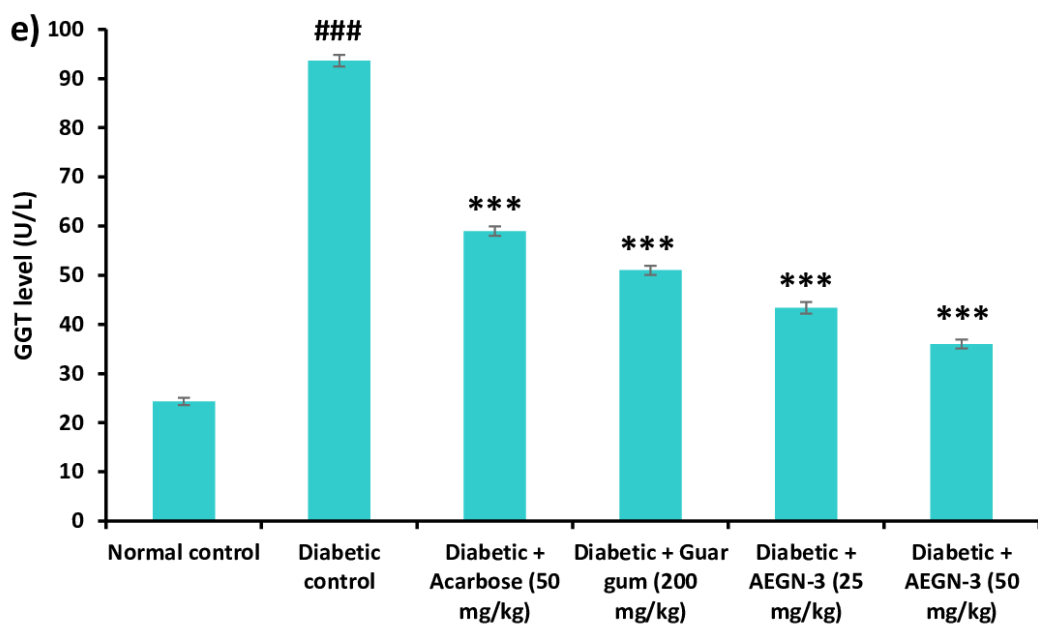
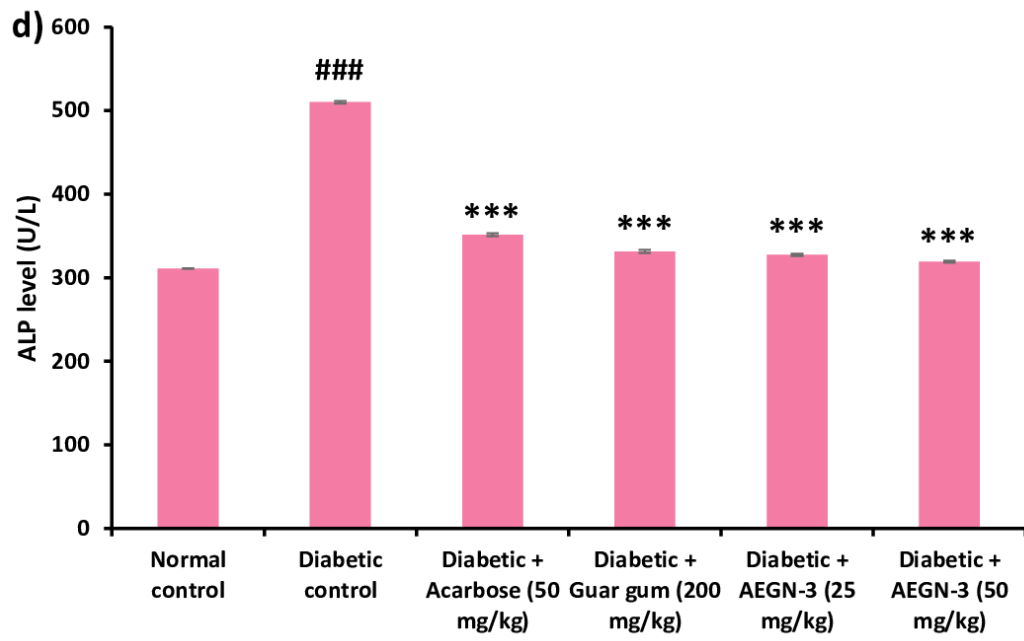
However, administration of acarbose and gaur gum (50 and 200 mg/kg) to untreated HFD-fed, STZ-induced diabetic rats resulted in a notable reduction in hepatic enzyme levels. Specifically,

bilirubin levels decreased by 2.09-fold ($p < 0.001$) and 2.36-fold ($p < 0.001$), SGOT levels by 1.39-fold ($p < 0.001$) and 1.54-fold ($p < 0.001$), and SGPT levels by 1.58-fold ($p < 0.001$) and 1.46-fold ($p < 0.001$), respectively. Similarly, ALP levels were reduced by 1.45-fold ($p < 0.001$) and 1.53-fold ($p < 0.001$), while GGT levels decreased by 1.58-fold ($p < 0.001$) and 1.83-fold ($p < 0.001$). LDH levels also followed this trend, showing a reduction of 1.58-fold ($p < 0.001$) and 1.83-fold ($p < 0.001$) with acarbose and guar gum treatment, respectively.

Conversely, treatment with AEGN-3 at doses of 25 mg/kg and 50 mg/kg resulted in a significant reduction in enzymatic levels. Specifically, bilirubin levels were reduced by 2.57-fold ($p < 0.001$) and 3.91-fold ($p < 0.001$), SGOT levels by 1.55-fold ($p < 0.001$) and 1.59-fold ($p < 0.001$), SGPT levels by 1.46-fold ($p < 0.001$) and 1.60-fold ($p < 0.001$), ALP levels by 1.55-fold ($p < 0.001$) and 1.59-fold ($p < 0.001$), and GGT levels by 2.16-fold ($p < 0.001$) and 2.60-fold ($p < 0.001$). LDH levels were similarly reduced by 2.16-fold ($p < 0.001$) and 2.60-fold ($p < 0.001$) at the respective doses. In conclusion, these findings indicate that administration of AEGN-3 at the higher dose of 50 mg/kg to HFD-fed, STZ-induced diabetic rats effectively enhances hepatic function by normalizing enzyme levels.







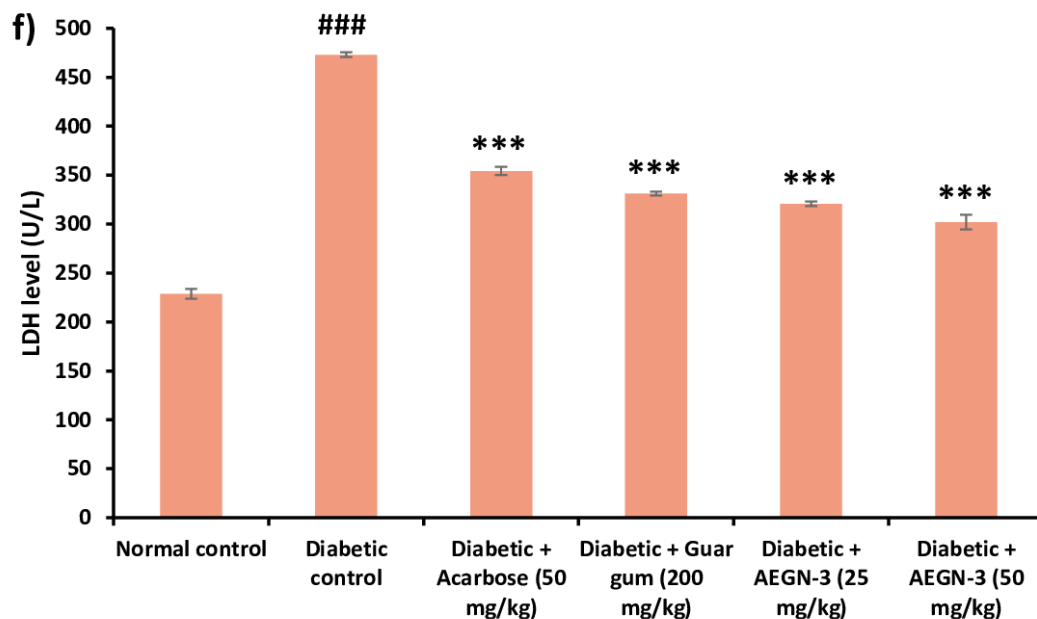


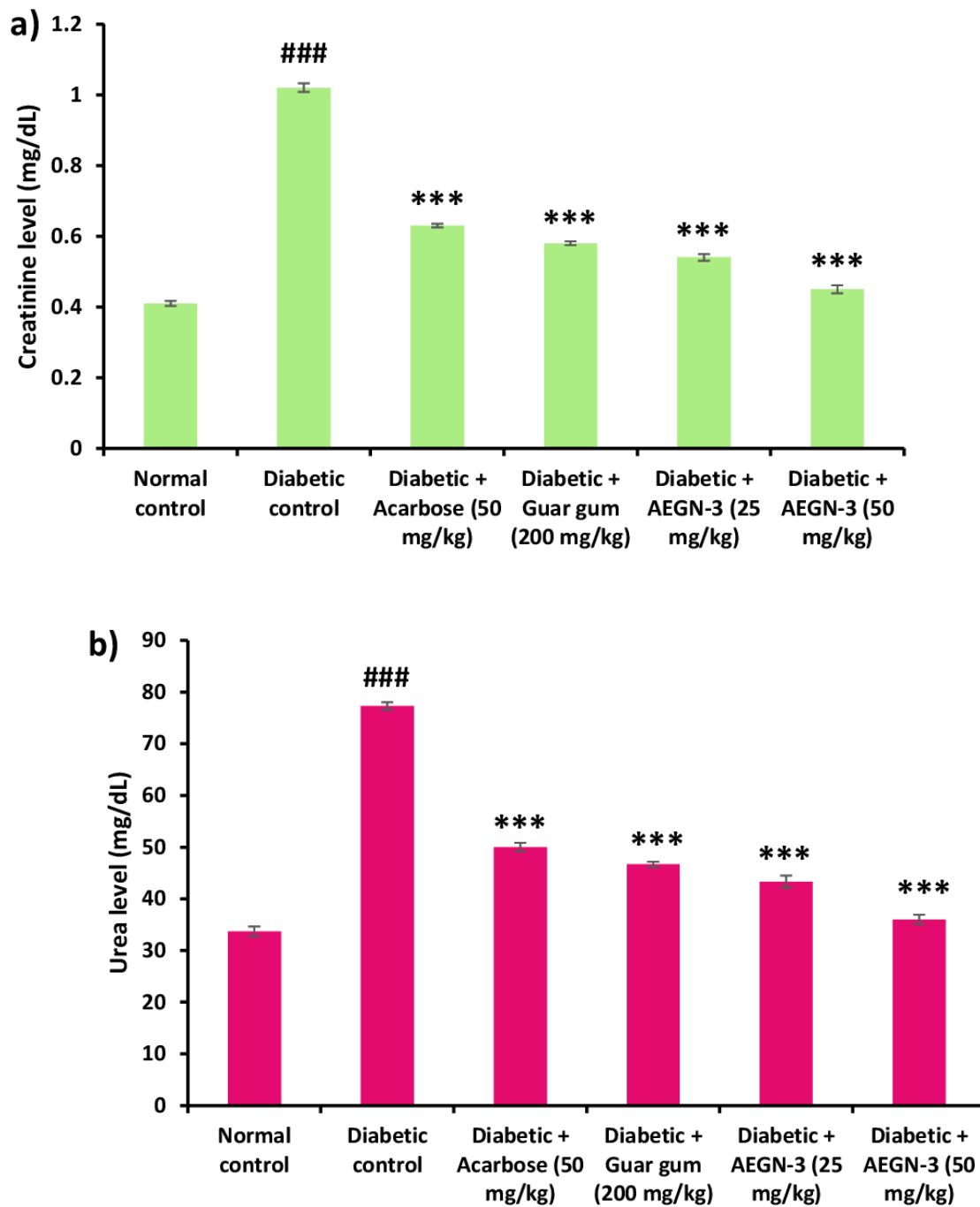
Figure 6.35: Effect of various treatments on serum liver function markers in diabetic rats. Panels represent serum levels of (a) bilirubin, (b) SGOT, (c) SGPT, (d) ALP, (e) GGT, and (f) LDH, measured at the end of the treatment period. Data are expressed as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared to normal control; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to diabetic control; ns denotes non-significant differences.

6.4.7 Administration of AEGN-3 reduces serum nephrotoxicity markers in diabetic rats

The results pertaining to serum nephrotoxic markers which are influenced under hyperglycemic conditions are detailed in Figure 6.36 a-c. As expected, HFD-fed, STZ-induced diabetic rats exhibited a significant increase in creatinine (2.48-fold; $p < 0.001$), urea (2.29-fold; $p < 0.001$), and uric acid (2.05-fold; $p < 0.001$), compared to the normal vehicle control group receiving a normal diet. However, after administering acarbose and guar gum at a dose of 50 mg/kg and 200 mg/kg to diabetic rats, there was a substantial decrease in the level of creatinine (1.61-fold; $p < 0.001$ and 1.75-fold; $p < 0.001$), urea (1.54-fold; $p < 0.001$ and 1.65-fold; $p < 0.001$), and uric acid (1.34-fold each; $p < 0.001$), compared to untreated diabetic rats.

Remarkably, the administration of AEGN-3 at a dose of 25 mg/kg and 50 mg/kg resulted in a significant reduction in the levels of these markers, i.e. 1.88-fold ($p < 0.001$) and 2.26-fold ($p < 0.001$) for creatinine, 1.59-fold ($p < 0.001$) and 1.75-fold ($p < 0.001$) for uric acid, and 1.78-fold ($p < 0.001$) and 2.14-fold ($p < 0.001$) for urea, respectively, when compared to untreated

diabetic rats. These findings collectively confirm that AEGN-3, at both dosages, effectively improves renal function markers by decreasing their levels in HFD-fed, STZ-induced diabetic animals; however, more significant effects were observed at the higher dose.



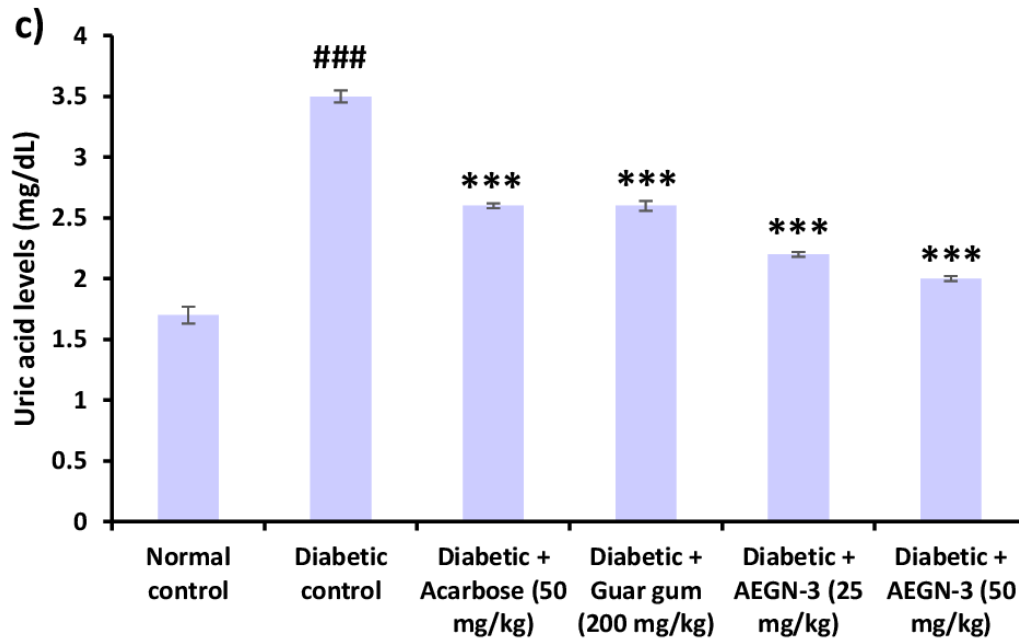


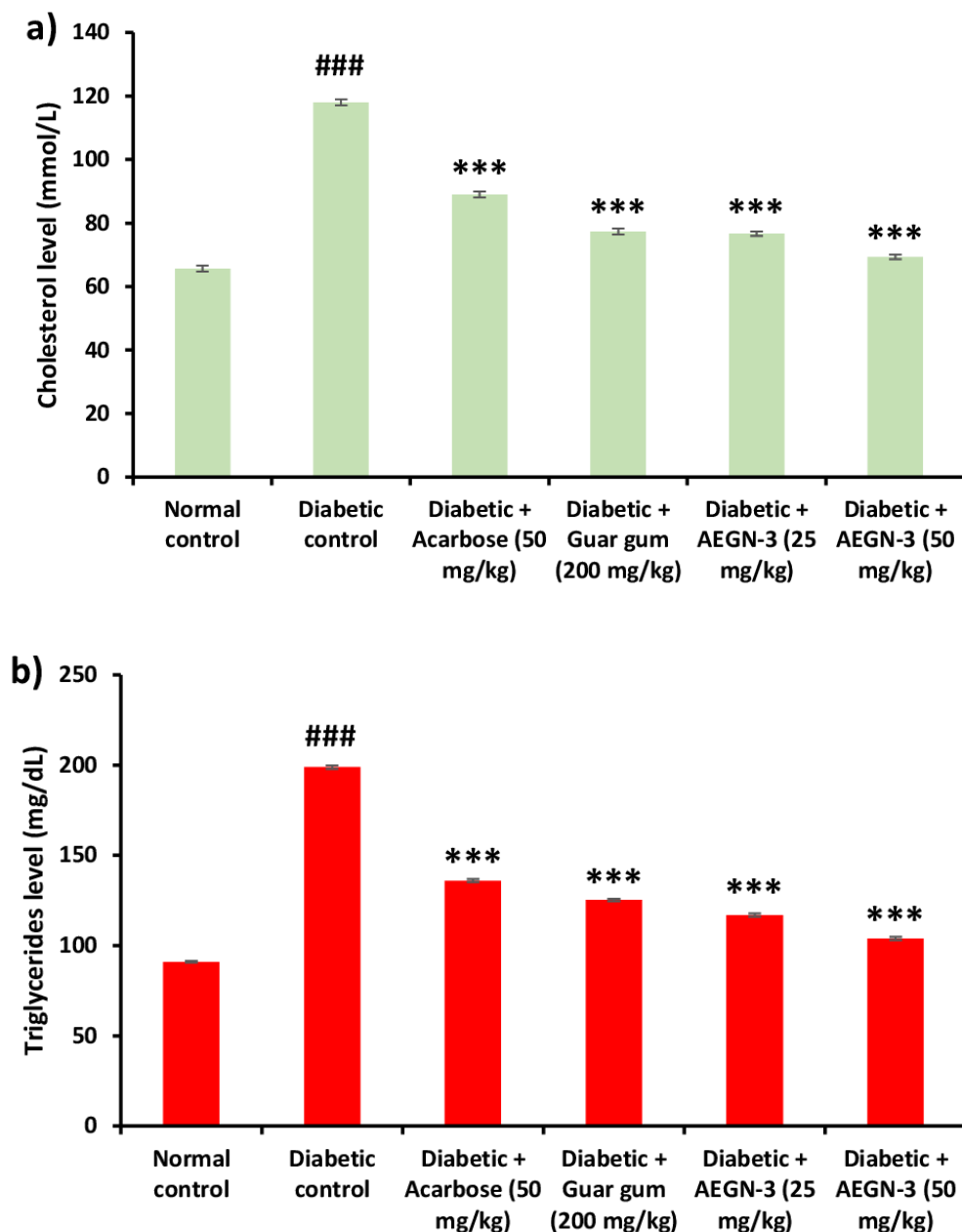
Figure 6.36: Effect of various treatments on serum renal function markers in diabetic rats. Panels represent serum levels of (a) creatinine, (b) urea, and (c) uric acid, measured at the end of the treatment period. Data are expressed as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared to normal control; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to diabetic control; ns denotes non-significant differences.

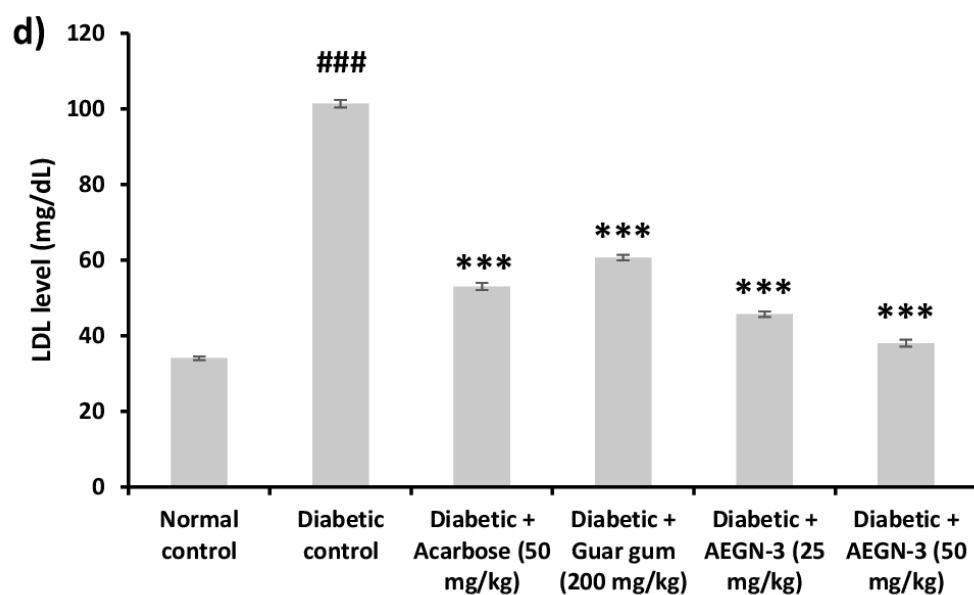
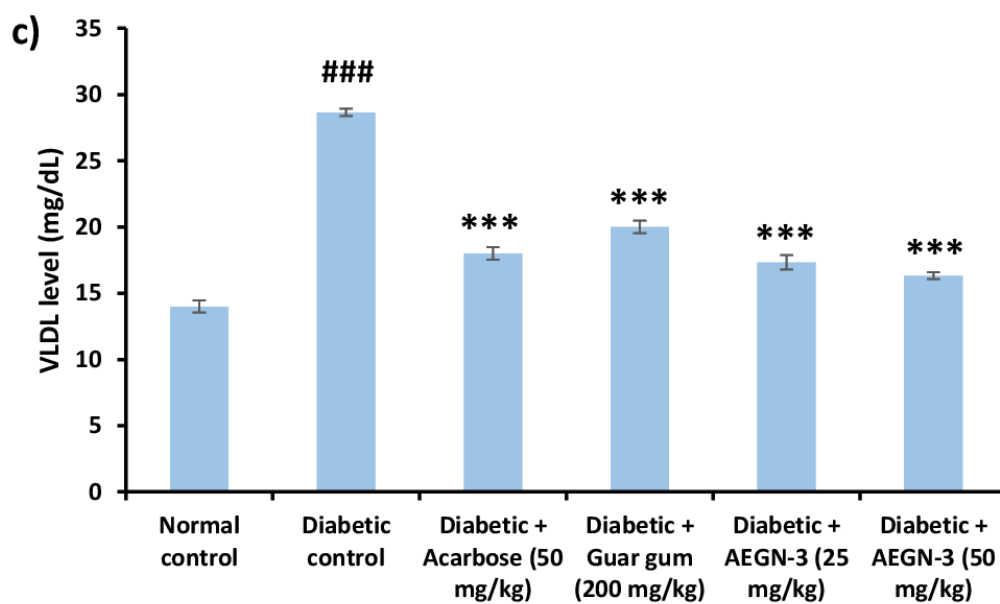
6.4.8 AEGN-3 administration ameliorates dyslipidemia and hepatic dysfunction in diabetic rats

HFD feeding followed by STZ administration induces pancreatic dysfunction, impairing the regulation of lipid metabolism and leading to excessive production of harmful lipids such as cholesterol, triglycerides, VLDL, and LDL, while simultaneously reducing protective HDL levels. Consequently, we sought to investigate the effects of AEGN-3 on lipid profile abnormalities in diabetic rats. As shown in Figure 6.37 a-e, untreated diabetic rats exhibited significant dyslipidemia, characterized by elevated cholesterol (1.79-fold; $p < 0.001$), triglycerides (2.18-fold; $p < 0.001$), VLDL (2.04-fold; $p < 0.001$), and LDL (2.98-fold; $p < 0.001$) levels, along with a marked reduction in HDL (3.27-fold decrease; $p < 0.001$), compared to non-diabetic controls.

However, treatment with acarbose (50 mg/kg) and guar gum (200 mg/kg) significantly improved the lipid profiles ($p < 0.001$), partially restoring normal metabolic function.

Moreover, these treatments also led to a notable reduction in hepatic enzyme levels, as evidenced by decreased bilirubin (2.09-fold and 2.36-fold; $p < 0.001$), SGOT (1.39-fold and 1.54-fold; $p < 0.001$), and SGPT (1.58-fold and 1.46-fold; $p < 0.001$) concentrations, respectively. Interestingly, AEGN-3 administration produced even greater improvements in lipid metabolism compared to standard treatments, significantly reducing cholesterol, triglycerides, VLDL, and LDL levels while concomitantly enhancing HDL concentrations ($p < 0.001$). Likewise, AEGN-3 treatment resulted in a more pronounced normalization of hepatic enzyme activities. Collectively, these findings suggest that AEGN-3 exerts a protective effect against dyslipidemia and liver dysfunction in HFD-fed, STZ-induced diabetic rats, thereby promoting overall metabolic health.





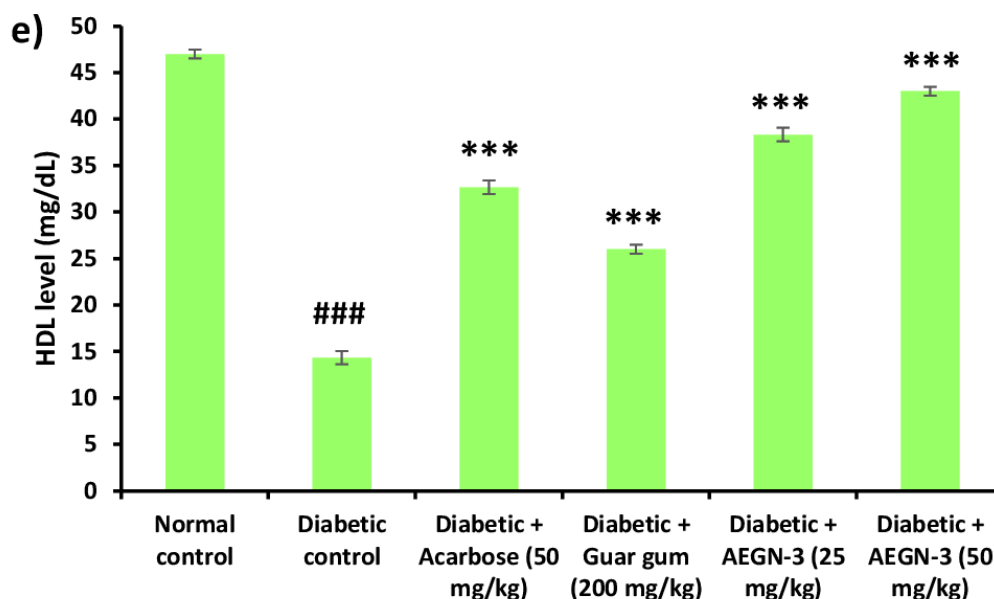


Figure 6.37: Effect of various treatments on serum hepatic function markers in diabetic rats. Panels represent serum levels of (a) cholesterol, (b) triglycerides, (c) VLDL, (d) LDL, and (e) HDL, measured at the end of the treatment period. Data are expressed as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared to normal control; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to diabetic control; ns denotes non-significant differences.

6.4.9 Histopathological assessment of hepatic, renal, pancreatic, and cardiac tissues following AEGN-3 administration

We conducted histopathological assessments of hepatic, renal, pancreatic, and cardiac tissues to evaluate the impact of AEGN-3 administration. These tissues were selected due to their crucial roles in the metabolic and organ-specific effects of diabetes. The following sections detail the histological changes observed in each organ following treatment with AEGN-3, highlighting the potential protective effects and restoration of tissue architecture.

6.4.9.1 AEGN-3 treatment preserves hepatic histoarchitecture in diabetic rats

The results pertaining to Figure 6.38 reveal that the histopathological analysis of liver tissues showed marked differences among the experimental groups. The normal control group exhibited well-preserved hepatic architecture, with uniformly arranged hepatocytes, clear cytoplasm, prominent nuclei, intact sinusoidal spaces, and a well-organized central vein, indicating normal hepatic morphology. However, the diabetic control group showed severe

hepatic injury, characterized by extensive hepatocellular degeneration, cytoplasmic vacuolization, sinusoidal dilation, inflammatory cell infiltration, and disruption of hepatic cords, reflecting significant structural disorganization due to persistent hyperglycemia. Consequently, diabetic rats treated with acarbose at 50 mg/kg dose displayed moderate improvement, with partial restoration of hepatic cord arrangement, reduction in cytoplasmic vacuolization, and improved sinusoidal integrity, although occasional hepatocyte swelling and mild inflammatory infiltration persisted. Likewise, diabetic rats treated with guar gum at 200 mg/kg demonstrated partial amelioration of liver damage, as evidenced by better organization of hepatocytes and reduced vacuolization, yet mild cellular disarray and inflammation remained noticeable.

In diabetic rats treated with AEGN-3 at a dose of 25 mg/kg, substantial recovery was observed, with more uniformly arranged hepatocytes, minimal cytoplasmic vacuolization, and improved sinusoidal preservation, accompanied by only slight inflammatory infiltration. Moreover, diabetic rats treated with AEGN-3 at a dose of 50 mg/kg exhibited near-complete restoration of hepatic architecture, with hepatocytes closely resembling the normal control group, showing well-organized hepatic lobules, clear cytoplasmic details, and intact sinusoidal spaces. Overall, these findings suggest that AEGN-3 treatment, particularly at 50 mg/kg, significantly mitigates diabetes-induced hepatic injury and promotes pronounced histological recovery in a dose-dependent manner.

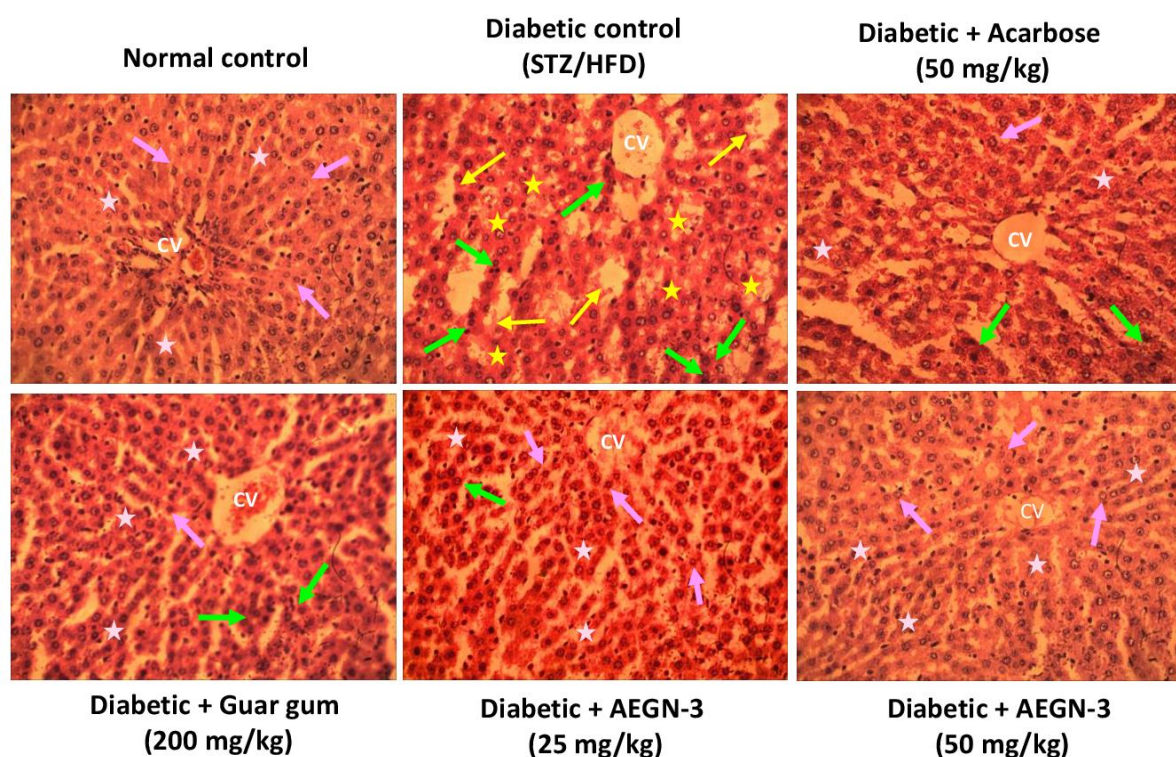


Figure 6.38: Histopathological analysis of liver tissues from different experimental groups stained with hematoxylin and eosin (H&E). Liver sections were examined at the end of the study to evaluate histological changes induced by diabetes and treatment interventions. CV indicates the central vein; yellow arrows indicate sinusoidal dilations; pink arrows denote normal hepatocytes; white stars represent intact sinusoidal spaces; yellow stars indicate cytoplasmic vacuolization; and green arrows point to areas of inflammation.

6.4.9.2 AEGN-3 treatment preserves renal histoarchitecture in diabetic rats

Histological analysis of kidney sections from the normal control group revealed well-preserved renal architecture, characterized by intact glomeruli and orderly arranged renal tubules without any signs of inflammation or structural damage. In contrast, the diabetic control group exhibited marked pathological alterations, including glomerular atrophy, glomerulosclerosis, tubular degeneration, and extensive interstitial inflammatory infiltration, indicating significant renal injury associated with diabetes. Treatment with acarbose in diabetic rats partially ameliorated renal damage, as evidenced by moderately preserved glomerular structure and reduced, but still noticeable, tubular disruption and inflammatory cell infiltration compared to the diabetic control group. Similarly, the diabetic group treated with guar gum at 200 mg/kg dose demonstrated comparable protective effects, with moderately restored glomerular and tubular morphology, although some pathological features persisted.

Remarkably, administration of the AEGN-3 at a dose of 25 mg/kg resulted in better histological improvements than acarbose or guar gum alone. Kidney sections from this group displayed relatively well-maintained glomeruli and tubules, with minimal inflammatory changes. Further enhancement was observed in the group treated with the AEGN-3 at 50 mg/kg, which showed nearly normal renal architecture, minimal tubular damage, and very limited inflammatory infiltration, closely resembling the histological appearance of the normal control group (Figure 6.39). These findings collectively suggest that the acarbose formulation, particularly at the higher dose, confers superior renoprotective effects against diabetes-induced kidney damage compared to acarbose and guar gum treatments.

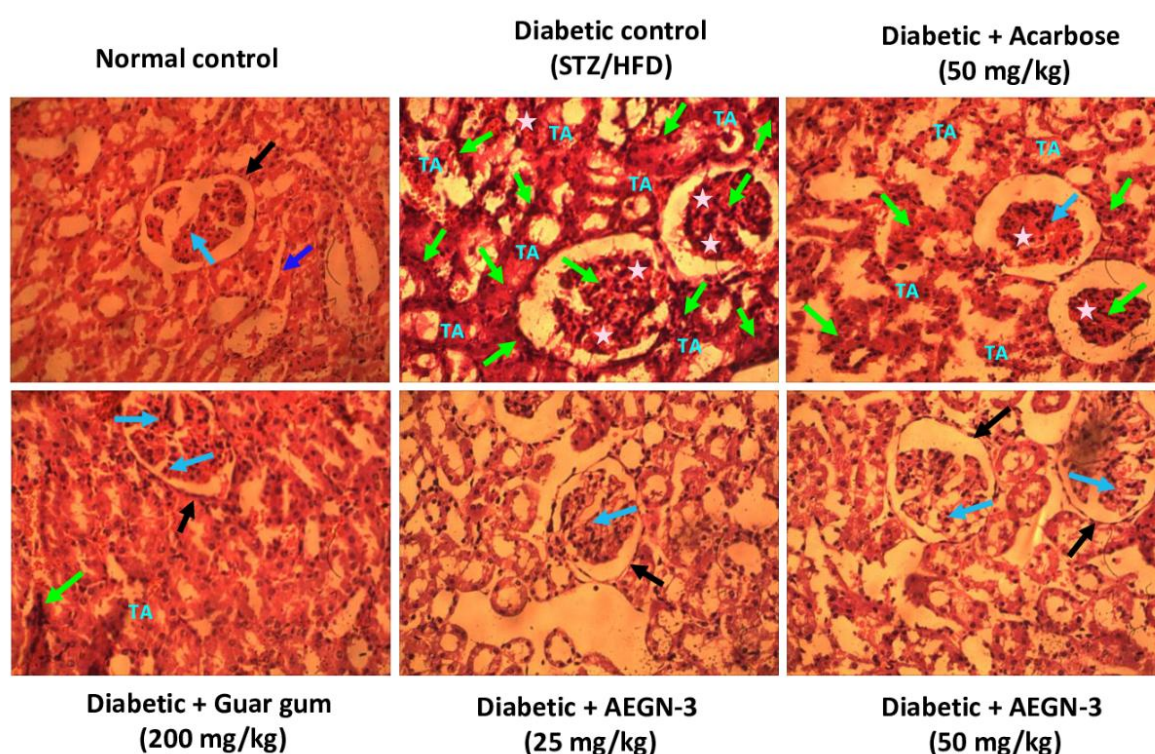


Figure 6.39: Histopathological analysis of renal tissues from different experimental groups stained with hematoxylin and eosin (H&E). Renal sections were examined at the end of the study to evaluate histological changes induced by diabetes and treatment interventions. TA indicates tubular atrophy; sky blue arrows denote intact glomeruli; black arrows indicate Bowman's capsules; blue arrows show renal tubules; green arrows highlight areas of inflammation; and stars represent glomerular damage.

6.4.9.3 AEGN-3 treatment preserves pancreatic histoarchitecture in diabetic rats

As shown in Figure 6.40, the histopathological evaluation of pancreatic tissue in the normal control group demonstrated well-preserved histoarchitecture, characterized by large, densely packed, and rounded islets of Langerhans surrounded by intact exocrine acini, with no signs of inflammatory infiltrates, cellular degeneration, or necrosis, indicative of optimal pancreatic functionality. In contrast, the diabetic control group exhibited profound pathological alterations, including marked atrophy and disorganization of the islets, extensive β -cell destruction, cytoplasmic vacuolization, widespread necrosis, and dense mononuclear inflammatory cell infiltration within both islet and acinar regions, confirming severe diabetes-induced pancreatic injury.

Treatment with acarbose conferred partial histological protection, as evidenced by moderately restored islet structure, a decrease in necrotic areas, and reduced, albeit persistent,

inflammatory cell infiltration. Administration of guar gum exhibited further histological improvement, characterized by relatively preserved islet morphology, moderate restoration of endocrine tissue, and attenuated inflammatory response compared to the diabetic control. Treatment with the AEGN-3 at 25 mg/kg dose revealed noticeable recovery of pancreatic architecture, with partially reconstructed islets, reduced necrosis, and diminished inflammatory infiltration, suggesting protective effects. Importantly, the 50 mg/kg dose of AEGN-3 displayed substantial histological preservation, with near-normal islet morphology, minimal inflammatory infiltration, decreased necrotic changes, and improved organization of exocrine tissue, reflecting significant β -cell regenerative potential and overall amelioration of diabetes-induced pancreatic damage.

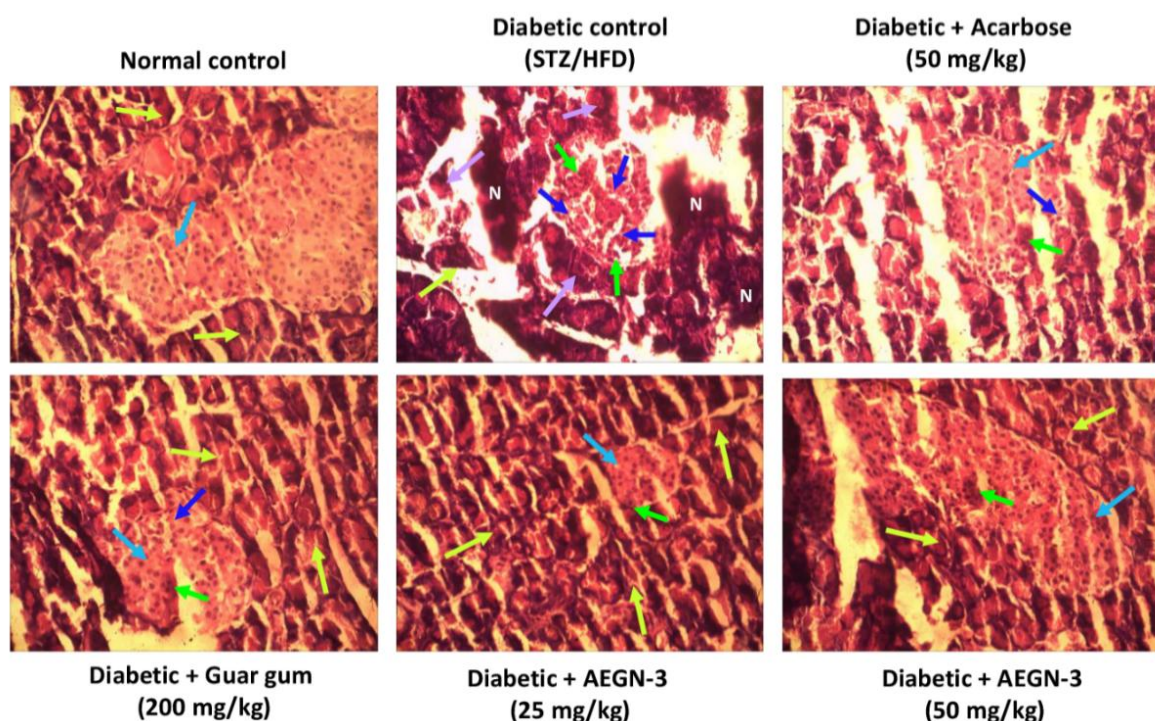


Figure 6.40: Histopathological analysis of pancreatic tissues from different experimental groups stained with hematoxylin and eosin (H&E). Pancreatic sections were examined at the end of the study to evaluate histological changes induced by diabetes and treatment interventions. N indicates necrosis; light green arrows indicate acinar cells; purple arrows denote atrophy and disorganization of the islets; blue arrows indicate cytoplasmic vacuolization; and bright green arrows point to areas of inflammation.

6.4.9.4 AEGN-3 treatment restores cardiac histoarchitecture in diabetic rats

In the non-diabetic healthy rat group, the heart tissue exhibited well-organized muscle fiber alignment, dense myocardium, and no significant intercellular spaces. The myocardial fibers appeared compact, with no evidence of fatty infiltration, vacuolation, or degeneration, confirming a normal histological architecture. In contrast, the diabetic untreated group displayed severe pathological changes, including extensive fragmentation of myocardial fibers, large clear vacuolated spaces, poor structural integrity, and significant degeneration, suggesting intense damage and severe disruption of cardiac tissue. There was also possible inflammatory infiltration, further indicating active tissue injury. The acarbose-treated diabetic group demonstrated moderate improvement, with a partial reduction in vacuolated spaces and slight restoration of myocardial fiber organization, though residual degeneration and fragmented areas were still apparent.

In the guar gum-treated group, there was evidence of moderate recovery, reflected by reduced vacuolization, better muscle fiber density, but some persistent inter-fiber spaces and mild degeneration remained. The low-dose formulation-treated group showed further improvements, with more densely packed myocardial fibers, less fragmentation, and decreased empty spaces, suggesting partial to significant recovery toward normal morphology. Notably, the high-dose formulation-treated group exhibited the most substantial recovery among treated groups, with remarkable muscle fiber realignment, dense myocardium, minimal vacuolation, and near-complete restoration of tissue integrity, approaching the histological features seen in healthy non-diabetic hearts (Figure 6.41).

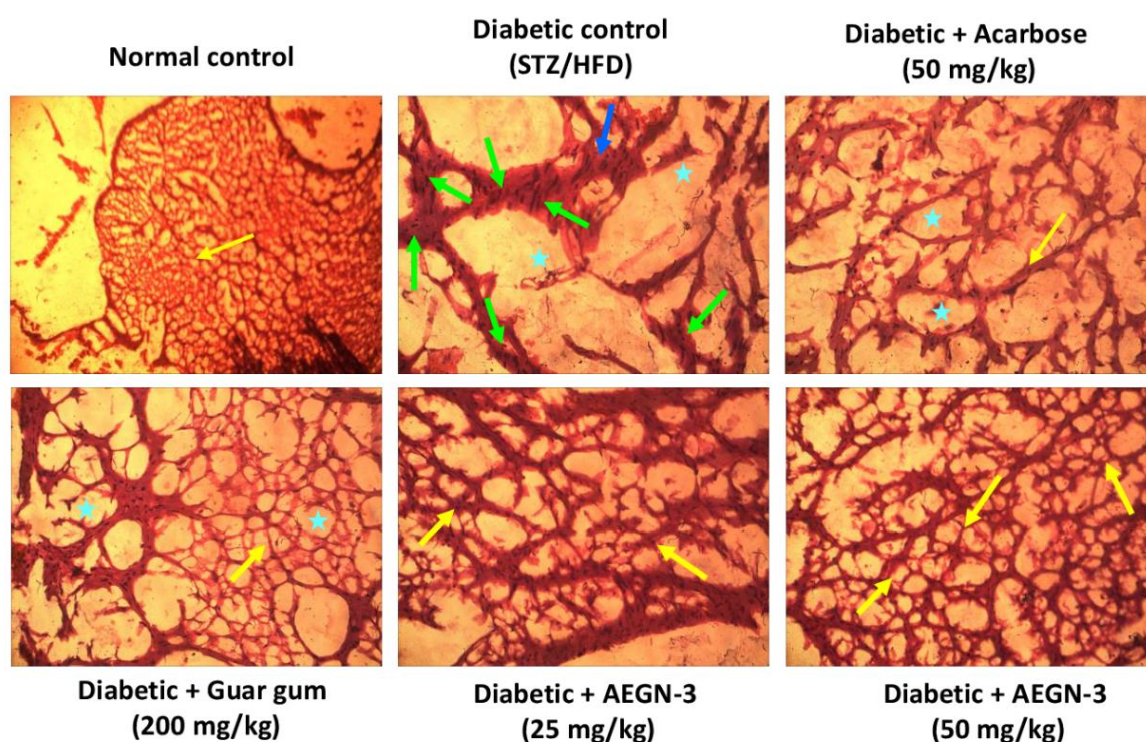


Figure 6.41: Histopathological analysis of cardiac tissues from different experimental groups stained with hematoxylin and eosin (H&E). Cardiac sections were examined at the end of the study to evaluate histological changes induced by diabetes and treatment interventions. Bright green arrows point to areas of inflammation; yellow arrows indicate well-organized muscle fibers; blue arrows show fragmentation of myocardial fibers; and stars represent vacuolated spaces.

6.4.10 AEGN-3 administration restores total protein levels in tissues of diabetic rats

Total protein content in tissue homogenates was quantified using the Lowry method, with BSA as the standard, to normalize antioxidant enzyme activities across groups. As shown in Figure 6.42, untreated diabetic rats exhibited a significant decline in total protein levels in liver, kidney, pancreas, and heart tissues compared to normal controls ($p < 0.001$), indicating hyperglycemia-induced metabolic stress and tissue damage.

Compared to untreated diabetic rats, treatment with Acarbose (50 mg/kg) and Guar gum (200 mg/kg) significantly restored protein content in all examined tissues ($p < 0.001$), indicating partial protection against diabetes-induced protein degradation. Notably, AEGN-3 (50 mg/kg) exhibited superior efficacy ($p < 0.001$), markedly restoring protein levels toward normal, which suggests potent cytoprotective and antioxidant effects, likely attributable to enhanced bioavailability and the synergistic action of the AEGN-3. Protein content values were

subsequently used to normalize antioxidant enzyme activities, expressed as units per mg protein.

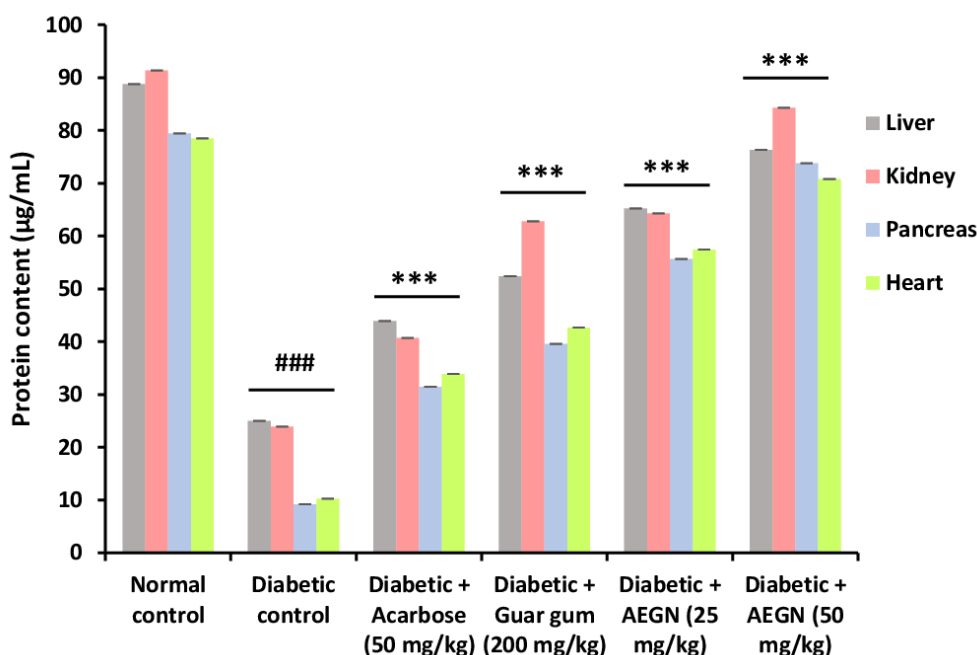


Figure 6.42: Effect of various treatments on tissue protein content in diabetic rats. Protein levels in the liver, kidney, pancreas, and heart were measured at the end of the experimental period and are expressed as $\mu\text{g/mL}$. Data are presented as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared with the normal control group; *** $p < 0.001$ compared with the diabetic control group.

6.4.11 AEGN-3 administration mitigates lipid peroxidative damage in HFD-fed, STZ-induced rat tissues

The results pertaining to Figure 6.43 show the impact of AEGN-3 on LPO levels in rats receiving different treatments. In diabetic rats, LPO levels were significantly elevated across all tissues compared to normal controls. The diabetic control group exhibited a nearly 6-fold increase in MDA levels in the liver, kidney, and 8-fold increase in pancreas ($p < 0.001$), with a nearly 8.5-fold increase in the heart ($p < 0.001$). Interestingly, treatment with acarbose at 50 mg/kg dose resulted in a moderate reduction in MDA levels, with a 1.45-fold decrease in the liver ($p < 0.001$), 1.46-fold reduction in the kidney ($p < 0.001$), and a 1.36-fold decrease in the pancreas ($p < 0.001$), while in the heart, MDA levels decreased by 1.39-fold ($p < 0.001$).

Conversely, guar gum at 200 mg/kg dose showed more substantial reductions in MDA levels, by 1.87-fold in the liver ($p < 0.001$), 2.01-fold in the kidney ($p < 0.001$), 1.80-fold in the pancreas ($p < 0.001$), and 1.85-fold in the heart ($p < 0.001$).

Notably, AEGN-3 at both doses significantly diminished MDA levels compared to acarbose and guar gum. Likewise, AEGN-3 at a dose of 25 mg/kg, notably reduced LPO, with a 2.54-fold reduction in the liver ($p < 0.001$), 2.49-fold in the kidney ($p < 0.001$), 2.45-fold in the pancreas ($p < 0.001$), and 2.39-fold in the heart ($p < 0.001$). Meanwhile, AEGN-3 at 50 mg/kg showed more substantial reductions in MDA levels, with a 3.28-fold decrease in the liver ($p < 0.001$), 3.85-fold reduction in the kidney ($p < 0.001$), 5.46-fold decrease in the pancreas ($p < 0.001$), and 4.31-fold reduction in the heart ($p < 0.001$), highlighting its superior antioxidant activity in all tissues. These results suggest that AEGN-3, especially at the higher dose, offers significant protection against oxidative damage in diabetic rats.

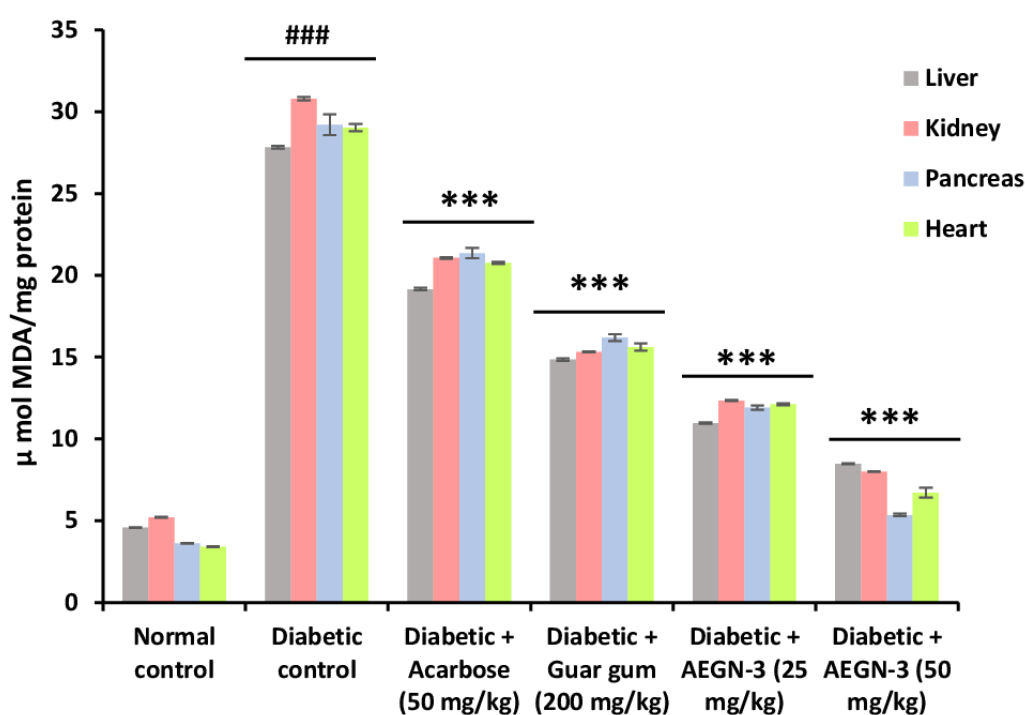


Figure 6.43: Effect of various treatments on MDA levels in diabetic rats. MDA, a marker of LPO, was measured in the liver, kidney, pancreas, and heart tissues at the end of the study and is expressed as $\mu\text{mol MDA/mg protein}$. Data are presented as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared to the normal control; *** $p < 0.001$ compared to the diabetic control.

6.4.12 AEGN-3 administration ameliorates the reduction of SOD activity in diabetic rat tissues

As expected, HFD-fed, STZ-induced diabetic rats showed a striking reduction in SOD levels across all tissues compared to normal controls (Figure 6.44). The SOD levels were reduced by 5.91-fold in the liver ($p < 0.001$), 10.29-fold in the kidney ($p < 0.001$), 5.38-fold in the pancreas ($p < 0.001$), and 3.88-fold in the heart ($p < 0.001$), indicating that HFD feeding followed by STZ administration induced oxidative stress. Interestingly, diabetic rats treated with acarbose at a dose of 50 mg/kg showed a moderate reduction in oxidative stress by enhancing SOD levels in the liver by 3.03-fold ($p < 0.001$), followed by the kidney (2.94-fold; $p < 0.001$), pancreas (2.63-fold; $p < 0.001$), and heart tissues (1.91-fold; $p < 0.001$). In contrast, administration of guar gum at a dose of 200 mg/kg exhibited superior effects in enhancing SOD levels in the liver (4.05-fold; $p < 0.001$), kidney (4.10-fold; $p < 0.001$), pancreas (3.48-fold; $p < 0.001$), and heart (2.08-fold; $p < 0.001$), compared to acarbose alone.

Among acarbose and guar gum formulations, administration of AEGN-3 at both doses demonstrated protective effects against HFD-fed, STZ-induced oxidative stress, as evidenced by increased SOD levels. AEGN-3 at a dose of 25 mg/kg showed a 4.66-fold increase in the liver ($p < 0.001$), 6.14-fold in the kidney ($p < 0.001$), 4.04-fold in the pancreas ($p < 0.001$), and 2.68-fold in the heart ($p < 0.001$). A more pronounced effect was observed with AEGN-3 at 50 mg/kg, resulting in a 5.29-fold increase in the liver ($p < 0.001$), 9.16-fold in the kidney ($p < 0.001$), 4.73-fold in the pancreas ($p < 0.001$), and 3.43-fold in the heart ($p < 0.001$). This significant increase in SOD levels highlights the protective effect of AEGN-3 against HFD-fed, STZ-induced oxidative stress in rats.

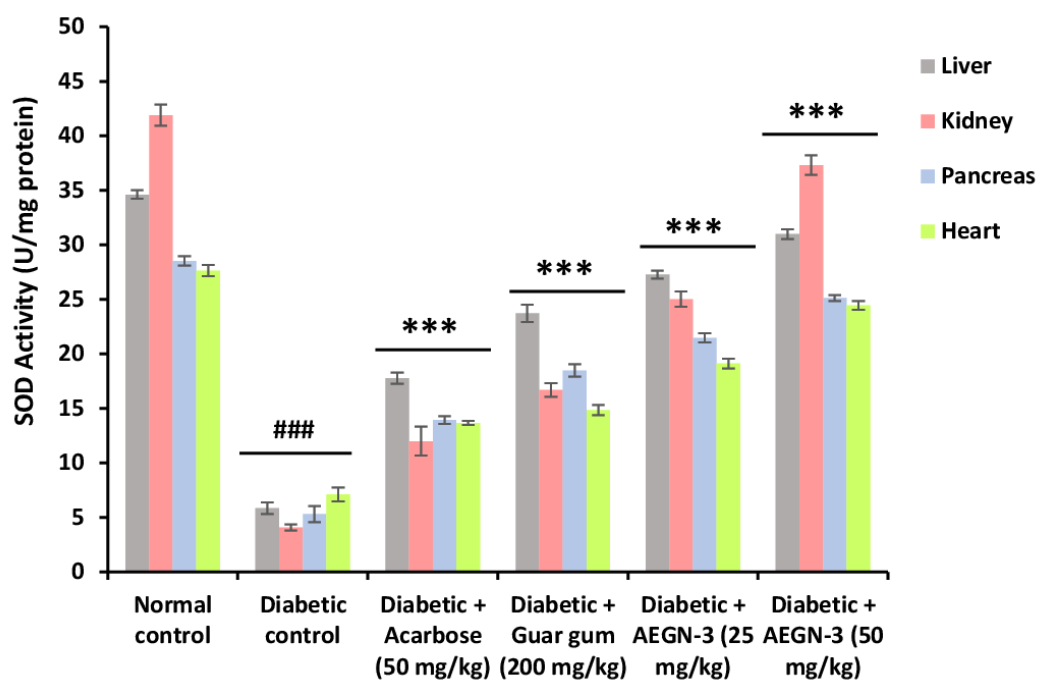


Figure 6.44: Effect of various treatments on SOD activity in diabetic rats. SOD activity was measured in the liver, kidney, pancreas, and heart tissues at the end of the study and is expressed as Units/mg protein. Data are presented as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared to the normal control; *** $p < 0.001$ compared to the diabetic control.

6.4.13 AEGN-3 treatment improves catalase activity in diabetic rats

Similar to SOD, GST activity was significantly reduced in the liver (4.05-fold; $p < 0.001$), kidney (5.20-fold; $p < 0.001$), pancreas (5.38-fold; $p < 0.001$), and heart (3.14-fold; $p < 0.001$) of HFD-fed, STZ-induced diabetic rats compared to normal controls. However, treatment with acarbose (50 mg/kg) and guar gum (200 mg/kg) notably improved catalase activity, with increases of 1.70-fold and 2.38-fold in the liver ($p < 0.001$), 2.26-fold and 2.66-fold in the kidney ($p < 0.001$), 2.92-fold and 3.95-fold in the pancreas ($p < 0.001$), and 1.51-fold and 1.80-fold in the heart ($p < 0.001$), respectively. While both treatments enhanced catalase activity, guar gum demonstrated a more pronounced reduction in oxidative stress compared to acarbose.

Importantly, administration of AEGN-3 at both doses further improved catalase activity beyond the effects observed with acarbose or guar gum alone. Specifically, AEGN-3 at 25 mg/kg and 50 mg/kg enhanced catalase activity by 2.92-fold and 3.60-fold in the liver ($p < 0.001$), 3.66-fold and 4.62-fold in the kidney ($p < 0.001$), 4.46-fold and 4.92-fold in the pancreas ($p < 0.001$),

and 2.37-fold and 2.72-fold in the heart ($p < 0.001$), respectively (Figure 6.45). These findings suggest that AEGN-3 treatment markedly enhances catalase activity and offers superior protection against oxidative stress in HFD-fed, STZ-induced diabetic rats compared to acarbose and guar gum.

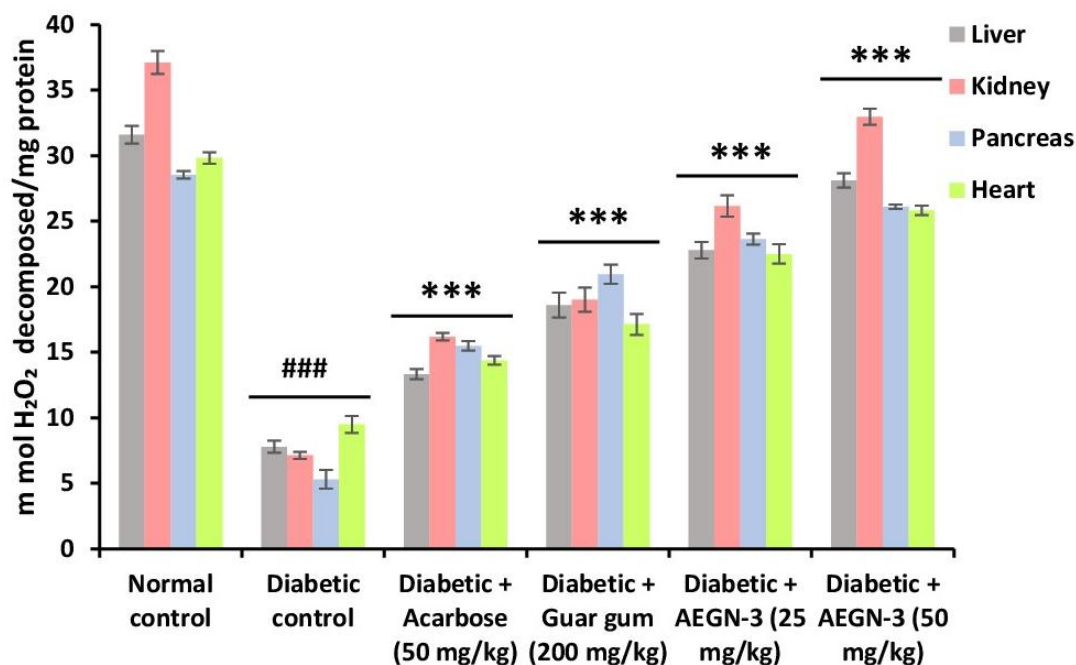


Figure 6.45: Effect of various treatments on catalase activity in diabetic rats. Catalase activity was measured in the liver, kidney, pancreas, and heart tissues at the end of the study and is expressed as mmol H₂O₂ decomposed/mg protein. Data are presented as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared to the normal control; *** $p < 0.001$ compared to the diabetic control.

6.4.14 AEGN-3 treatment restores GST activity in diabetic rats

As shown in Figure 6.46, GST activity was significantly reduced across all examined tissues in untreated HFD-fed, STZ-induced diabetic rats compared to normal controls. The diabetic control group demonstrated an approximate 4-fold decrease in GST activity in the liver ($p < 0.001$), a 5-fold decrease in the kidney ($p < 0.001$), a 3.19-fold decrease in the pancreas ($p < 0.001$), and a 3.85-fold decrease in the heart ($p < 0.001$). Treatment with acarbose (50 mg/kg) resulted in a moderate improvement in GST activity, with increases of 1.99-fold in the liver ($p < 0.001$), 1.60-fold in the kidney ($p < 0.001$), 1.68-fold in the pancreas ($p < 0.001$), and 1.82-fold in the heart ($p < 0.001$). In contrast, guar gum (200 mg/kg) showed a more substantial

enhancement, increasing GST activity by 2.52-fold in the liver ($p < 0.001$), 2.42-fold in the kidney ($p < 0.001$), 2.18-fold in the pancreas ($p < 0.001$), and 2.29-fold in the heart ($p < 0.001$).

Notably, AEGN-3 treatment at both 25 mg/kg and 50 mg/kg doses demonstrated superior efficacy compared to both acarbose and guar gum. Administration of AEGN-3 resulted in a significant increase in GST activity, with 3.71-fold and 4.39-fold improvements in the liver ($p < 0.001$), 3.90-fold and 4.75-fold in the kidney ($p < 0.001$), 2.48-fold and 2.94-fold in the pancreas ($p < 0.001$), and 2.69-fold and 3.50-fold in the heart ($p < 0.001$). These findings highlight the potential of AEGN-3 to effectively restore GST activity and mitigate oxidative stress associated with HFD-fed, STZ-induced diabetes.

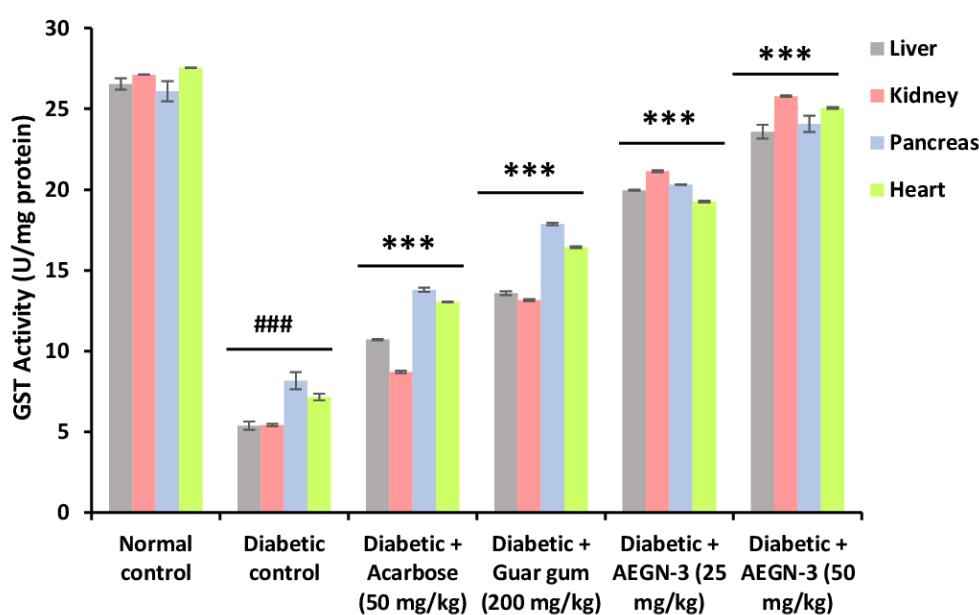


Figure 6.46: Effect of various treatments on GST activity in diabetic rats. GST activity was measured in the liver, kidney, pancreas, and heart tissues at the end of the study and is expressed as Units/mg protein. Data are presented as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared to the normal control; *** $p < 0.001$ compared to the diabetic control.

6.4.15 AEGN-3 treatment attenuates xanthine oxidase activity in diabetic rat tissues

In untreated diabetic rats, xanthine oxidase activity was significantly elevated across all tissues compared to normal controls. The diabetic control group exhibited a nearly 23-fold increase in xanthine oxidase activity in the liver, 27-fold increase in the kidney, 17.83-fold increase in the pancreas ($p < 0.001$), and nearly 18-fold increase in the heart ($p < 0.001$). Interestingly,

treatment with acarbose at 50 mg/kg resulted in a moderate reduction in xanthine oxidase activity, with a 1.80-fold decrease in the liver ($p < 0.001$), 1.57-fold reduction in the kidney ($p < 0.001$), 1.08-fold decrease in the pancreas ($p < 0.001$), and a 1.36-fold reduction in the heart ($p < 0.001$). Conversely, guar gum at 200 mg/kg showed more substantial reductions in xanthine oxidase activity, with a 2.42-fold reduction in the liver ($p < 0.001$), 2.87-fold in the kidney ($p < 0.001$), 1.33-fold in the pancreas ($p < 0.001$), and 1.94-fold in the heart ($p < 0.001$). Notably, AEGN-3 treatment at both doses significantly diminished xanthine oxidase activity compared to acarbose and guar gum. AEGN-3 at 25 mg/kg dose significantly reduced xanthine oxidase activity, with a 4.97-fold decrease in the liver ($p < 0.001$), 3.78-fold reduction in the kidney ($p < 0.001$), 2.21-fold reduction in the pancreas ($p < 0.001$), and a 2.18-fold decrease in the heart ($p < 0.001$). Likewise, AEGN-3 at 50 mg/kg showed the most substantial reductions in xanthine oxidase activity, with a 7.77-fold decrease in the liver ($p < 0.001$), 8.35-fold reduction in the kidney ($p < 0.001$), 9.73-fold decrease in the pancreas ($p < 0.001$), and a 13.24-fold reduction in the heart ($p < 0.001$), highlighting its superior antioxidant activity in all tissues (Figure 6.47). These results suggest that AEGN-3, particularly at the higher dose, offers significant protection against xanthine oxidase-induced oxidative damage in diabetic rats.

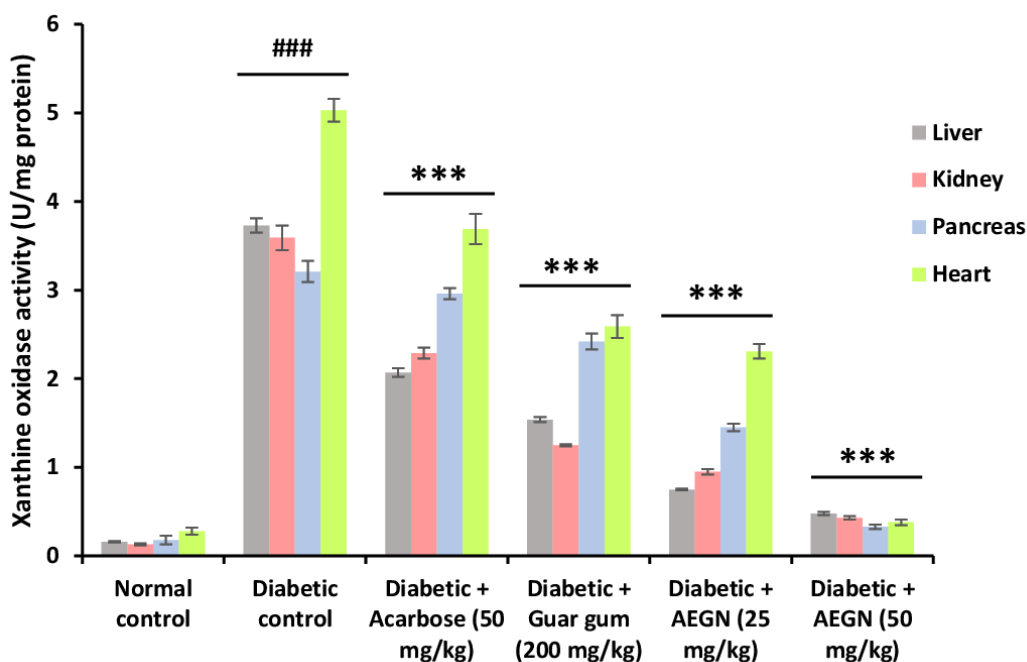


Figure 6.47: Effect of various treatments on xanthine oxidase activity in diabetic rats. Xanthine oxidase activity was measured in the liver, kidney, pancreas, and heart tissues at the end of the study and is expressed as Units/mg protein. Data are presented as mean $\pm$ SEM ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by

Tukey's multiple comparison post hoc test. Statistical significance is indicated as #### $p < 0.001$ compared to the normal control; *** $p < 0.001$ compared to the diabetic control.

6.4.16 AEGN-3 administration attenuates the elevation of acetylcholinesterase activity in diabetic rat tissues

Figure 6.48 illustrates the effect of AEGN-3 on AChE activity in diabetic rats. In untreated HFD-fed, STZ-induced diabetic rats, AChE activity was significantly elevated in all examined tissues compared to normal controls. The diabetic control group exhibited a nearly 10.5-fold increase in AChE activity in the liver ($p < 0.001$), 7.76-fold increase in the kidney ($p < 0.001$), 19.10-fold increase in the pancreas ($p < 0.001$), and nearly 15.45-fold increase in the heart ($p < 0.001$). Treatment with acarbose at 50 mg/kg resulted in a moderate reduction in AChE activity, with a 1.64-fold decrease in the liver ($p < 0.001$), 1.52-fold reduction in the kidney ($p < 0.001$), 1.56-fold decrease in the pancreas ($p < 0.001$), and 1.70-fold reduction in the heart ($p < 0.001$). Conversely, guar gum at 200 mg/kg showed more substantial reductions in AChE activity, with a 2.15-fold reduction in the liver ($p < 0.001$), 2.62-fold reduction in the kidney ($p < 0.001$), 2.03-fold reduction in the pancreas ($p < 0.001$), and 2.14-fold reduction in the heart ($p < 0.001$).

Notably, AEGN-3 treatment at both doses significantly diminished AChE activity compared to acarbose and guar gum. AEGN-3 at 25 mg/kg significantly reduced AChE activity, with a 2.96-fold decrease in the liver ($p < 0.001$), 3.84-fold reduction in the kidney ($p < 0.001$), 3.78-fold reduction in the pancreas ($p < 0.001$), and 4.03-fold decrease in the heart ($p < 0.001$). Likewise, AEGN-3 at 50 mg/kg showed the most substantial reductions in AChE activity, with 8.47-fold decrease in the liver ($p < 0.001$), 6.71-fold reduction in the kidney ($p < 0.001$), 11.14-fold decrease in the pancreas ($p < 0.001$), and 9.21-fold reduction in the heart ($p < 0.001$), highlighting its superior effect on AChE inhibition in all tissues. These results suggest that AEGN, particularly at the higher dose, offers significant protection against AChE-induced damage in diabetic rats.

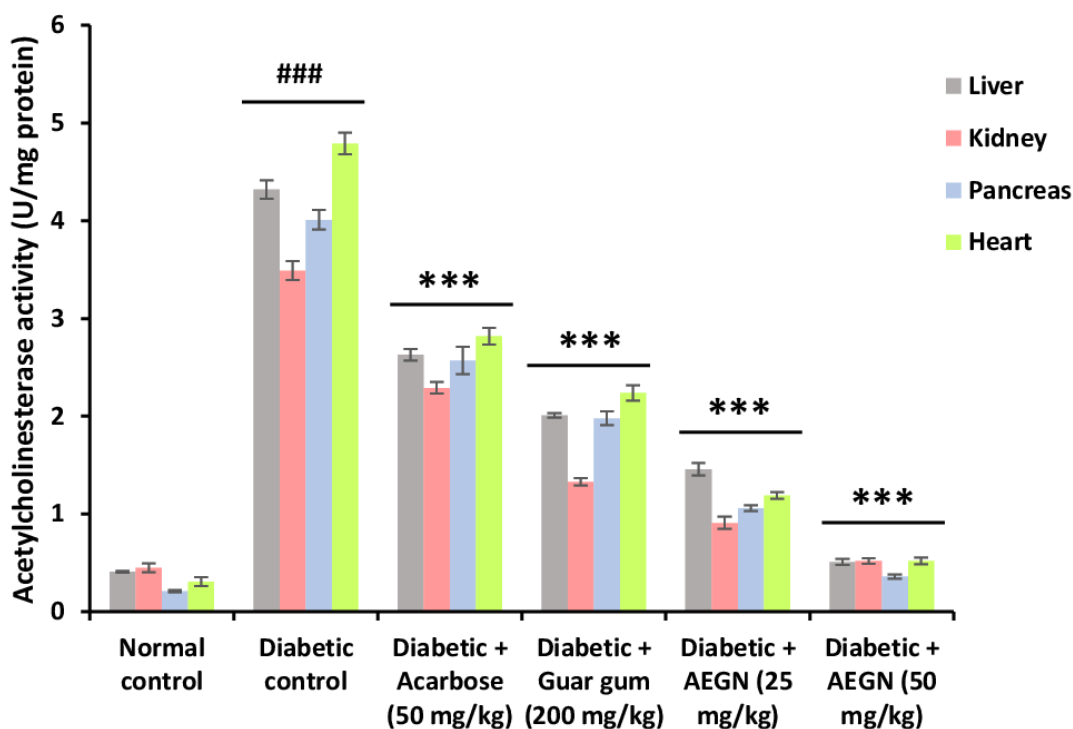


Figure 6.48: Effect of various treatments on AChE activity in diabetic rats. AChE activity was measured in the liver, kidney, pancreas, and heart tissues at the end of the study and is expressed as Units/mg protein. Data are presented as mean $\pm$ SEM (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ###p < 0.001 compared to the normal control; ***p < 0.001 compared to the diabetic control.

6.4.17 AEGN-3 attenuates TNF- α -mediated inflammation in tissues of diabetic rats

Since hyperglycemia is closely associated with inflammation due to the overexpression of pro-inflammatory genes such as TNF- α , it is critical to analyze the expression of these markers in the tissues of diabetic animals following various treatments. As depicted in Figure 6.49, HFD-fed, STZ-induced diabetic rats exhibited significant dysregulation of TNF- α , as evidenced by a marked increase in the liver (3.70-fold, p < 0.001), kidney (3.12-fold, p < 0.001), pancreas (5.19-fold, p < 0.001), and heart (4.65-fold, p < 0.001), indicating heightened inflammation and compromised anti-inflammatory defenses in the tissues of untreated diabetic rats.

Treatment with acarbose and guar gum at doses of 50 mg/kg and 200 mg/kg, respectively, significantly improved the inflammatory profile. Acarbose reduced TNF- α levels in the liver (2.03-fold and 1.83-fold, p < 0.001), kidney (1.79-fold and 1.62-fold, p < 0.001), pancreas (2.02-fold and 1.79-fold, p < 0.001), and heart (1.81-fold and 1.55-fold, p < 0.001), indicating

an enhanced anti-inflammatory response compared to untreated diabetic controls. Importantly, AEGN-3 at both doses produced a remarkable reduction in TNF- α levels across all tissues. At 25 mg/kg, AEGN-3 led to a 2.35-fold reduction in the liver ($p < 0.001$), 2.11-fold in the kidney ($p < 0.001$), 2.33-fold in the pancreas ($p < 0.001$), and 2.44-fold in the heart ($p < 0.001$). This effect was even more pronounced at the 50 mg/kg dose, with reductions of 2.84-fold in the liver ($p < 0.001$), 2.80-fold in the kidney ($p < 0.001$), 3.65-fold in the pancreas ($p < 0.001$), and 3.69-fold in the heart ($p < 0.001$). These findings indicate that AEGN-3 at both doses notably decreases inflammation and protects tissues from HFD-fed, STZ-induced inflammatory damage.

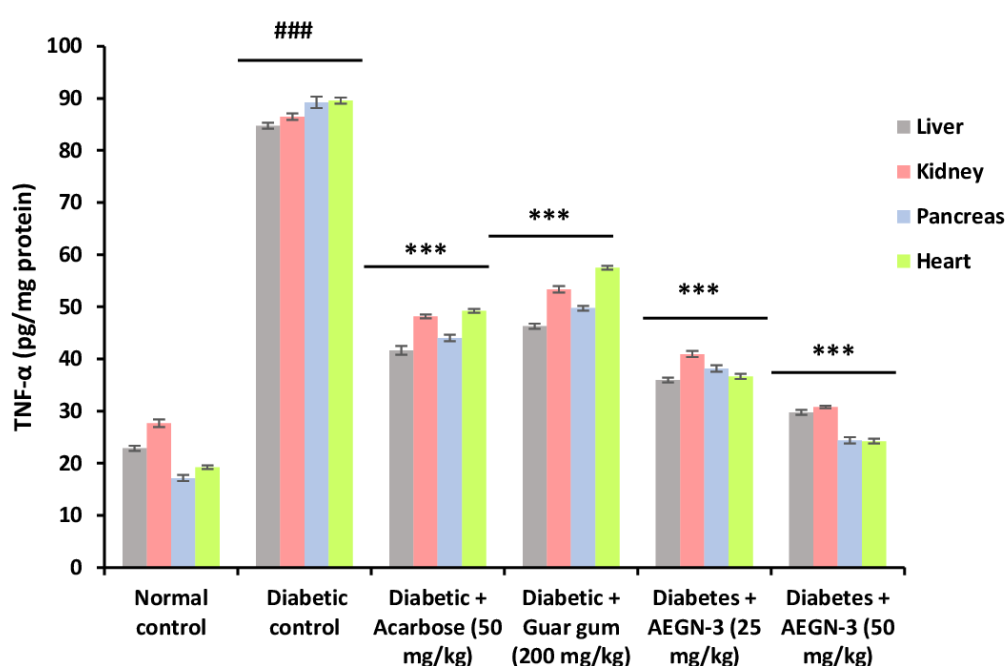


Figure 6.49: Effect of various treatments on TNF- α in diabetic rats. TNF- α level was measured in the liver, kidney, pancreas, and heart tissues at the end of the study and were measured in picograms per milligram of protein (pg/mg protein). Data are presented as mean $\pm$ SEM ($n = 2$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared to the normal control; *** $p < 0.001$ compared to the diabetic control.

6.4.18 Administration of AEGN-3 significantly attenuated IL-6-mediated inflammation in the tissues of diabetic rats

Consistent with the trend observed for TNF- α , a significant increase in IL-6 levels was also noted in the liver (4.62-fold; $p < 0.001$), kidney (2.62-fold; $p < 0.001$), pancreas (4.56-fold; p

< 0.001), and heart (5.94-fold; $p < 0.001$) of HFD-fed, STZ-induced diabetic rats compared to the normal control group. This elevation in IL-6 expression further supports the presence of inflammation associated with diabetes in this model. Consequently, treatment with acarbose and guar gum at doses of 50 mg/kg and 200 mg/kg, respectively, led to a significant improvement in the inflammatory profile across all examined tissues. Specifically, administration of acarbose and guar gum reduced IL-6 levels by 1.94-fold and 1.42-fold in the liver ($p < 0.001$), 1.50-fold and 1.28-fold in the kidney ($p < 0.001$), 1.81-fold and 1.58-fold in the pancreas ($p < 0.001$), and 1.72-fold and 1.59-fold in the heart ($p < 0.001$), respectively. These findings indicate that both treatments effectively mitigated IL-6-mediated inflammation, although the extent of reduction varied.

Moreover, AEGN-3 treatment demonstrated a more pronounced anti-inflammatory effect compared to acarbose and guar gum. At a 25 mg/kg dose, AEGN-3 significantly decreased IL-6 levels in the liver (2.27-fold; $p < 0.001$), kidney (1.62-fold; $p < 0.001$), pancreas (2.02-fold; $p < 0.001$), and heart (2.35-fold; $p < 0.001$). However, a further enhancement in anti-inflammatory efficacy was observed at the 50 mg/kg dose, with reductions of 3.99-fold in the liver ($p < 0.001$), 2.16-fold in the kidney ($p < 0.001$), 3.51-fold in the pancreas ($p < 0.001$), and 3.99-fold in the heart ($p < 0.001$). Similarly to its effect on TNF- α , AEGN-3 therapy substantially attenuated IL-6 levels across all tissues, suggesting a broad anti-inflammatory potential (Figure 6.50). Notably, even at the lower dose, AEGN-3 exhibited greater efficacy compared to conventional treatments, highlighting its therapeutic promise in mitigating diabetes-associated inflammation.

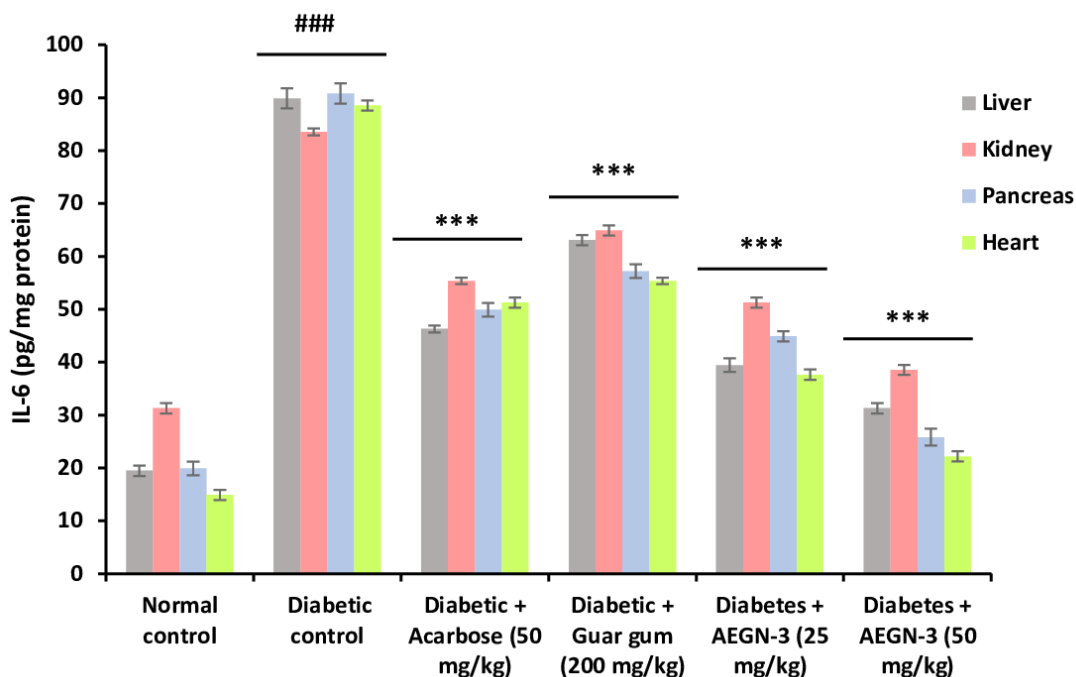


Figure 6.50: Effect of various treatments on IL-6 in diabetic rats. IL-6 level was measured in the liver, kidney, pancreas, and heart tissues at the end of the study and were measured in picograms per milligram of protein (pg/mg protein). Data are presented as mean $\pm$ SEM (n = 2). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ###p < 0.001 compared to the normal control; ***p < 0.001 compared to the diabetic control.

6.4.19 AEGN-3 restores IL-10-mediated anti-inflammatory responses in the tissues of diabetic rats

As shown in Figure 6.51, normal control rats exhibited higher IL-10 levels, reflecting an intact anti-inflammatory response. However, in HFD-fed, STZ-induced diabetic rats, IL-10 levels were significantly reduced, indicating compromised inflammatory regulation. Specifically, IL-10 levels decreased by 4.05-fold in the liver (p < 0.001), 3.70-fold in the kidney (p < 0.001), 5.26-fold in the pancreas (p < 0.001), and 5.27-fold in the heart (p < 0.001), compared to normal controls. Consequently, this reduction in IL-10 levels highlights the profound inflammatory imbalance induced by diabetes.

To counteract this reduction, diabetic rats were treated with acarbose (50 mg/kg) and guar gum (200 mg/kg) that showed a notable improvement. Treatment with acarbose and guar gum significantly elevated IL-10 levels in the liver (2.38-fold and 2.08-fold; p < 0.001), kidney (2.66-fold and 2.02-fold; p < 0.001), pancreas (3.21-fold and 2.36-fold; p < 0.001), and heart

(3.22-fold and 2.89-fold; $p < 0.001$), respectively, compared to untreated diabetic rats. Interestingly, while both acarbose and guar gum demonstrated protective effects, AEGN-3 exhibited an even greater ability to restore IL-10 levels. Specifically, administration of AEGN-3 at 25 mg/kg and 50 mg/kg resulted in a substantial enhancement of IL-10 levels by 3.33-fold and 3.79-fold in the liver ($p < 0.001$), 2.82-fold and 3.29-fold in the kidney ($p < 0.001$), 3.82-fold and 4.59-fold in the pancreas ($p < 0.001$), and 4.00-fold and 4.85-fold in the heart ($p < 0.001$), respectively. These findings indicate that AEGN-3 not only restores IL-10 expression more effectively than conventional treatments but also offers significant protection against diabetes-associated inflammatory dysregulation.

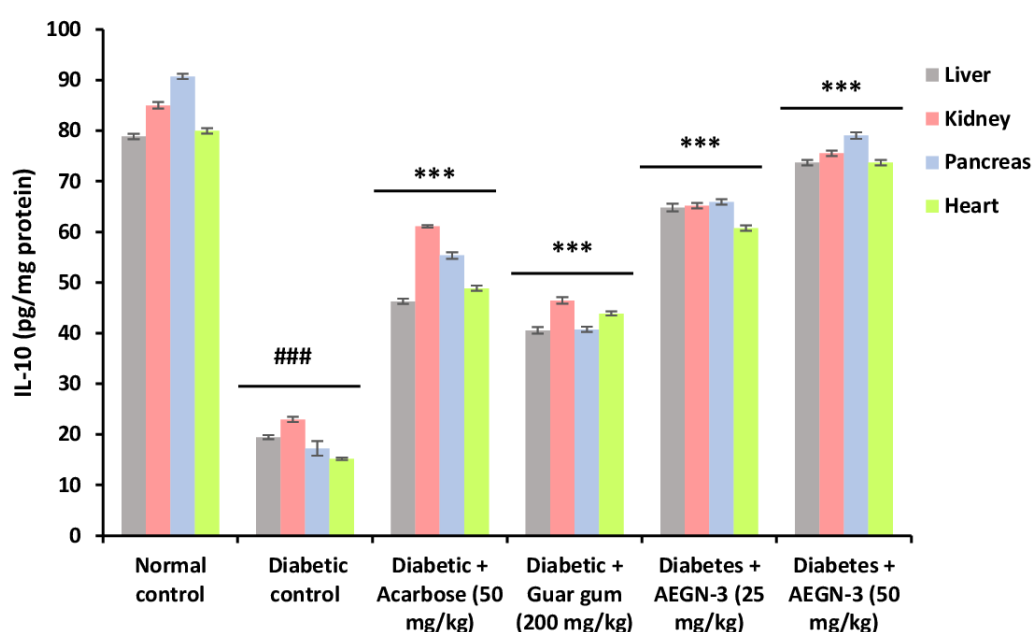


Figure 6.51: Effect of various treatments on IL-10 in diabetic rats. IL-10 level was measured in the liver, kidney, pancreas, and heart tissues at the end of the study and were measured in picograms per milligram of protein (pg/mg protein). Data are presented as mean $\pm$ SEM ($n = 2$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Statistical significance is indicated as ### $p < 0.001$ compared to the normal control; *** $p < 0.001$ compared to the diabetic control.

6.5 Network pharmacology results

6.5.1 Analysis between targets of acarbose and type 2 diabetes

In this study, the targets of acarbose were predicted using SwissTarget Prediction (<http://www.swisstargetprediction.ch/>), STITCH (<http://stitch.embl.de/>), BindingDB (<https://www.bindingdb.org/bind/index.jsp>), PharmMapper

(<http://lilab.ecust.edu.cn/pharmmapper/>), and CTD (<https://ctdbase.org/>), which resulted in the prediction of different 427 genes. This gene set was designated as the "targets of acarbose". Furthermore, the targets of type 2 diabetes were predicted using OMIM, DISGENET, GeneCards, and MalaCards, collectively providing a total of 3,710 genes, which are henceforth designated as the "targets of type 2 diabetes". After completing the retrieval process, duplicate entries were removed, and the remaining entries were normalized using UniProt (<https://www.uniprot.org/>), resulting in a final list of 127 genes (Figure 6.52).

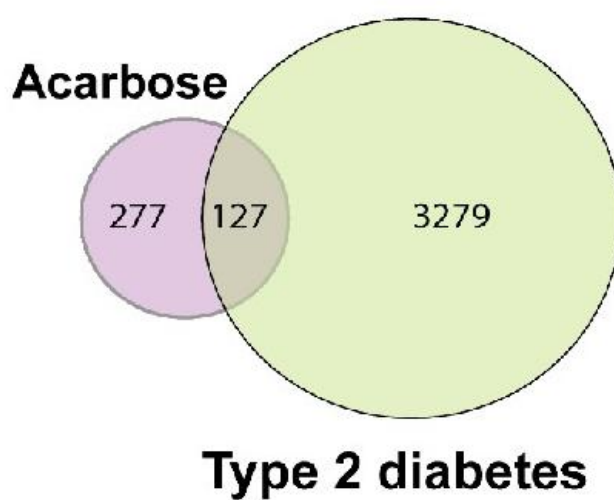


Figure 6.52: Venn diagram showing the intersection between acarbose-related targets and type 2 diabetes-associated genes, identifying 127 overlapping targets.

6.5.2 Functional enrichment and pathways of acarbose in type 2 diabetes

These common targets were subjected to GO and KEGG pathway enrichment analyses to explore their functional relevance. GO enrichment analysis revealed that these targets were significantly involved in biological processes such as glucose metabolic process, insulin receptor signaling, PI3K/AKT pathway regulation, and inflammatory responses (Figure 6.53a). In the cellular component category, enriched terms included mitochondria, cytosol, insulin receptor complex, and plasma membrane suggesting subcellular sites critical for insulin signaling and glucose transport (Figure 6.53b). The molecular function analysis indicated significant enrichment in protein kinase activity, insulin receptor binding, glucose transporter activity, and cytokine activity (Figure 6.53c), highlighting the targets' regulatory role in signal transduction and metabolic control. KEGG pathway analysis further revealed that the overlapping targets were enriched in several type 2 diabetes-relevant signaling pathways, including insulin resistance, adipocytokine signaling, AMPK signaling, AGE-RAGE signaling,

and lipid and glucose metabolism (Figure 6.53d). These results suggest that acarbose may exert its antidiabetic effects via multi-target modulation of critical metabolic and signaling cascades.

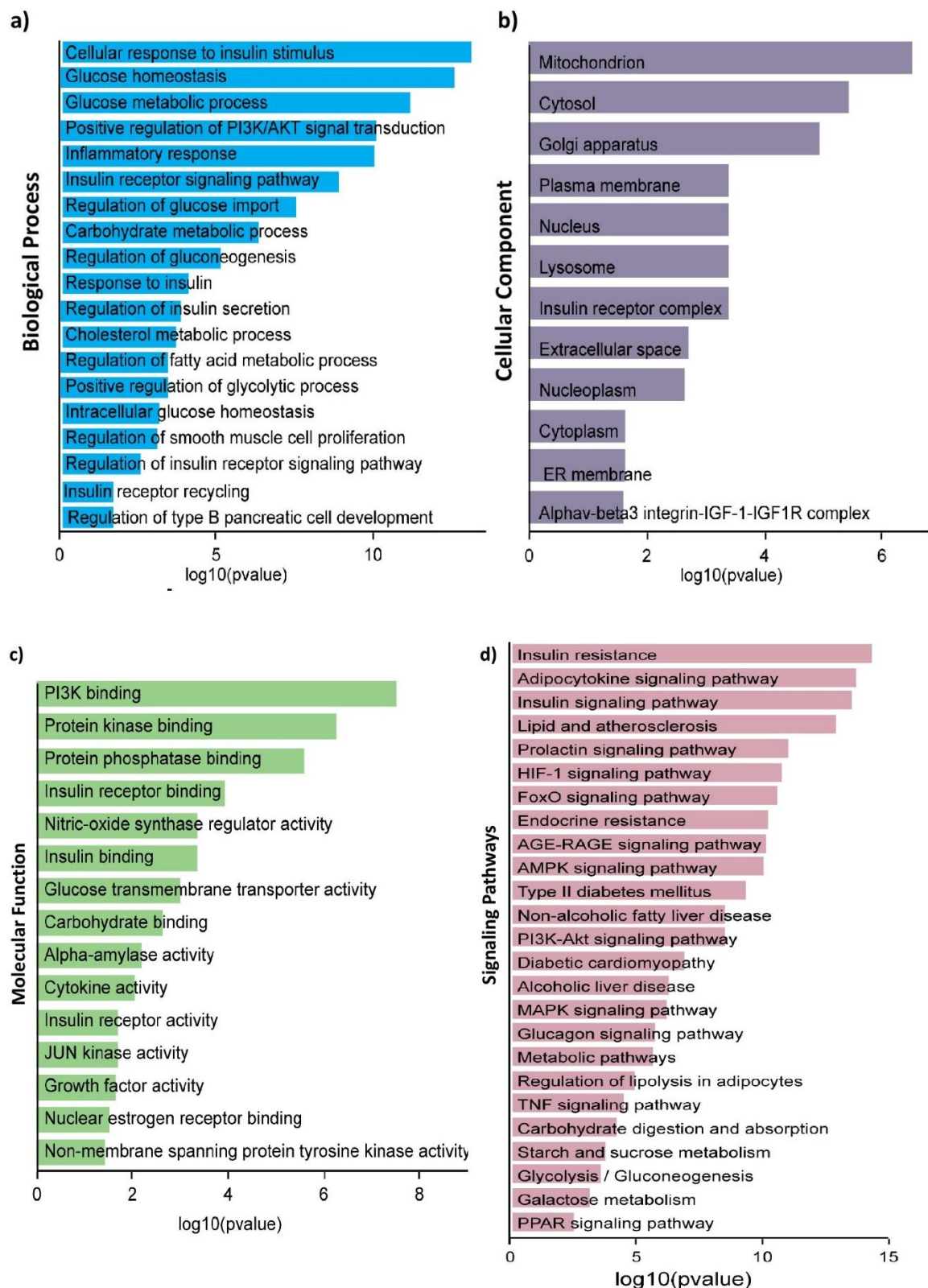


Figure 6.53: Gene Ontology (GO) enrichment analysis of the overlapping targets categorized into: **(a)** Biological Process enriched processes, **(b)** Cellular Component enriched localizations, **(c)** Molecular Function significantly enriched functions include insulin receptor binding, protein kinase binding, and glucose transporter activity, and **(d)** KEGG pathway enrichment analysis showing key type 2 diabetes-associated pathways.

6.5.3 Network analysis reveals key molecular targets

Protein regulatory network analysis of the shared targets identified highly interconnected hub proteins, many of which are key regulators of insulin signaling and inflammation (Figure 6.54a). Topological analysis highlighted major hub genes such as TNF, EGFR, STAT3, AKT1, PPARG, and ESR1, which may serve as central nodes in the acarbose-mediated therapeutic network (Figure 6.54b). Collectively, these findings suggest that acarbose modulates a complex network of proteins involved in metabolic regulation, inflammation, and signal transduction, thereby offering therapeutic benefit in type 2 diabetes through multi-target mechanisms.

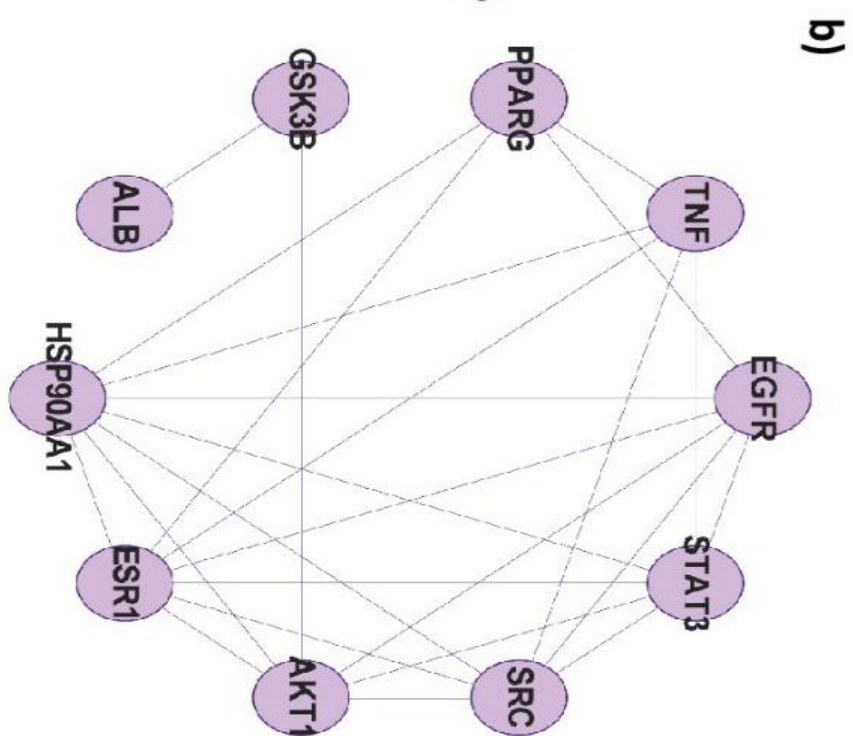
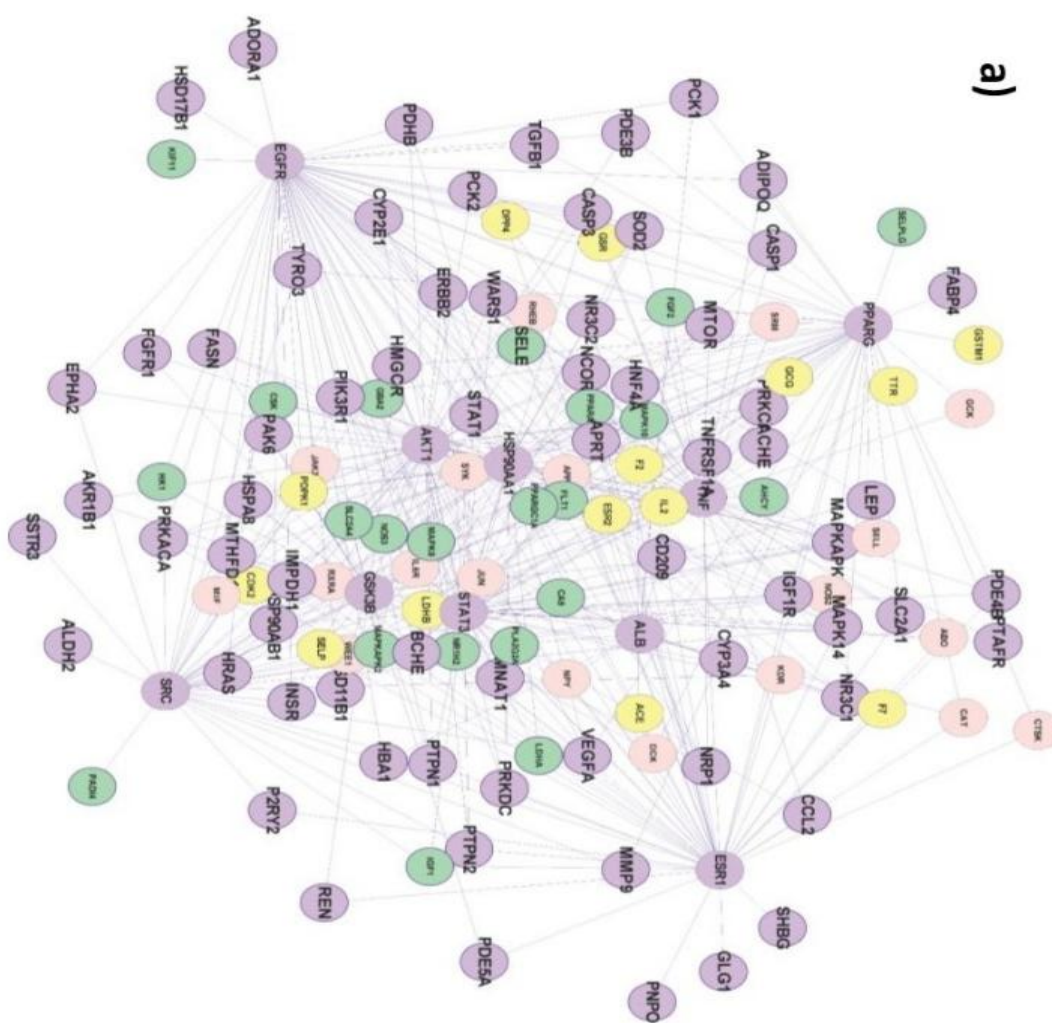


Figure 6.54: Network analysis of overlapping targets and identification of key hub genes. **(a)** Protein-protein interaction (PPI) network of the overlapping targets, constructed using GeneMania and visualized in Cytoscape; hub genes are highlighted by node size and connectivity, and **(b)** Subnetwork of key hub genes identified using network topology analysis, showing major regulatory genes including TNF, EGFR, STAT3, AKT1, SRC, ESR1, GSK3B, HSP90AA1, and PPARG.

6.5.4 Genotype phenotype association and functional characterization of core targets

To identify genotype-phenotype associations, 127 genes were analyzed using VarElect, and the top 10 disease-related proteins were selected based on Cytoscape analysis. SRC, a proto-oncogenic tyrosine kinase, exhibited the highest disease association likelihood among the analyzed targets, with a probability exceeding 92%, and is involved in insulin secretion by regulating glucokinase localization in pancreatic β -cells. AKT1 followed closely, with over 90% predicted involvement, playing a central role in glucose uptake via GLUT4 translocation, its dysfunction contributes to insulin resistance. GSK3B showed an association probability above 83%, impairing insulin signaling through IRS-1 phosphorylation and regulating gluconeogenesis and lipid metabolism. Albumin (ALB), with a contribution of more than 82%, serves as a crucial indicator of diabetic nephropathy, where hyperglycemia leads to glomerular damage and albuminuria, raising cardiovascular risks.

HSP90AA1, linked with a nearly 80% association, is implicated in insulin receptor instability and oxidative stress under hyperglycemic conditions. STAT3, with close to 79% likelihood, drives chronic inflammation and β -cell dysfunction through IL-6-induced SOCS3 expression and apoptosis. EGFR demonstrated over 77% relevance by modulating insulin sensitivity through survival and proliferation signaling pathways. TNF showed about 70% involvement in promoting insulin resistance via chronic inflammation and serine phosphorylation of insulin receptors. ESR1, contributing nearly 69%, supports insulin sensitivity and glucose regulation, with its impairment leading to metabolic disruption, especially in postmenopausal women. Lastly, PPARG, with a disease-association score of approximately 58%, plays a critical role in adipocyte differentiation and lipid metabolism; where, dysfunction results in insulin resistance and ectopic fat accumulation. Together, these proteins act as key regulators of insulin signaling, inflammation, and metabolic homeostasis, making them valuable therapeutic targets in managing type 2 diabetes.

6.5.5 Comparative expression analysis of core target genes across metabolic and peripheral tissues in type 2 diabetes

Different regulatory patterns are revealed by the expression analysis of important genes in peripheral and metabolic tissues (Figure 6.55). In liver tissue, type 2 diabetes samples exhibited a significant increase in AKT1 expression compared to normal samples ($p < 0.05$). Similarly, STAT3 expression was significantly elevated in type 2 diabetes liver samples ($p < 0.05$). No statistically significant differences were observed between normal and type 2 diabetes samples for GSK3 β , ALB, ESR1, EGFR, HSP90AA1, PPARG, SRC, or TNF (ns). In the pancreas, expression levels of AKT1, GSK3 β , ALB, ESR1, EGFR, HSP90AA1, PPARG, SRC, TNF, and STAT3 did not differ significantly between normal and type 2 diabetes samples (ns), indicating preserved transcriptional profiles under diabetic conditions. In adipose tissue, GSK3 β expression was significantly higher in type 2 diabetes samples compared to normal samples ($p < 0.05$), whereas EGFR expression was significantly reduced in type 2 diabetes samples ($p < 0.05$). The remaining genes, including AKT1, ALB, ESR1, HSP90AA1, PPARG, SRC, TNF, and STAT3, showed no significant differences between normal and type 2 diabetes samples (ns).

In skeletal muscle, AKT1 expression was significantly elevated ($p < 0.01$) and PPARG expression was significantly decreased ($p < 0.01$) in type 2 diabetes samples compared to normal samples, while GSK3 β , ALB, ESR1, EGFR, HSP90AA1, SRC, TNF, and STAT3 remained unchanged (ns). In peripheral blood samples collected from normal and type 2 diabetes experimental groups, GSK3 β expression was significantly increased ($p < 0.01$), whereas ESR1 ($p < 0.05$) and HSP90AA1 ($p < 0.05$) were significantly reduced in type 2 diabetes samples compared to normal samples. No significant differences were observed for AKT1, ALB, EGFR, PPARG, SRC, TNF, or STAT3 (ns). Collectively, these findings demonstrate that type 2 diabetes is associated with tissue-specific gene expression changes, with distinct regulatory patterns observed across metabolic organs and circulation when compared with normal samples. These observations reveal the intricate tissue-specific regulatory networks that are responsible for metabolic dysfunctions in type 2 diabetes.

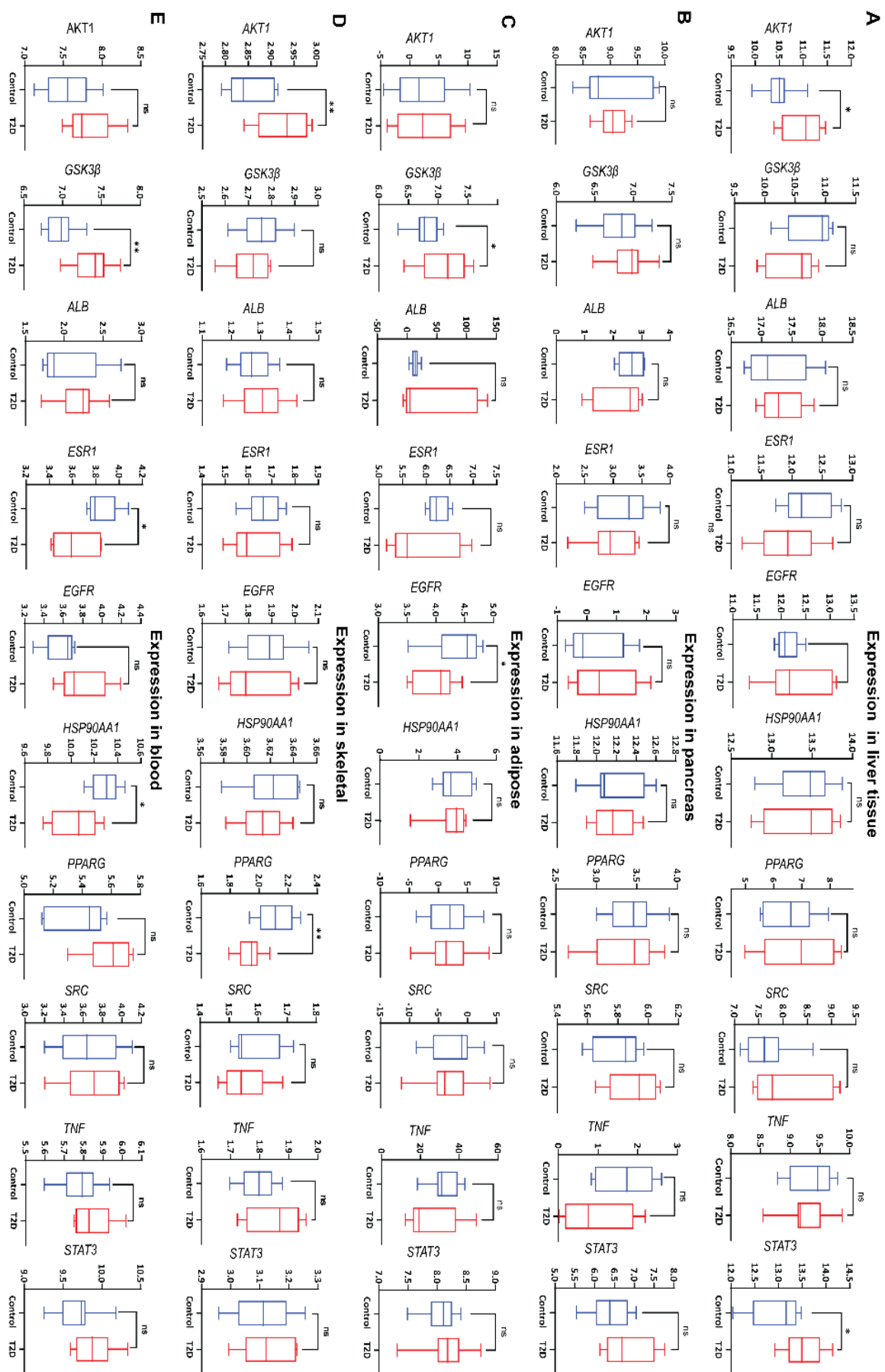


Figure 6.55: Differential expression of core target genes in control and type 2 diabetes samples across multiple tissues: (a) liver (GSE15653), (b) pancreas (GSE20966), (c) adipose tissue (GSE29226), (d) skeletal muscle (GSE55650), and (e) peripheral blood (GSE15932). Blue boxes represent control samples and red boxes represent type 2 diabetes samples. Statistical significance between groups is indicated as ns (not significant), * $p < 0.05$, and ** $p < 0.01$.

6.5.6 Molecular docking reveals stable binding of acarbose to hub proteins

To investigate the binding affinity and interaction patterns of acarbose with key regulatory proteins in type 2 diabetes, molecular docking simulations were performed for the top 10 hub genes identified from the PPI network. Figure 6.56 (a-j) illustrates the 3D surface representations of the protein-ligand complexes and their corresponding 2D interaction maps, highlighting the molecular contacts formed between acarbose and the protein binding pockets. Acarbose was shown to bind stably within the active or allosteric sites of all ten targets AKT1, ALB, EGFR, ESR1, GSK3B, HSP90AA, PPARG, SRC, STAT3, and TNF. To elucidate the binding mode of acarbose to targets of acarbose against type 2 diabetes, molecular docking studies were performed. The docking interactions predominantly involved hydrogen bonds, van der Waals forces, and hydrophobic contacts, contributing to strong ligand-receptor stability. Several residues were consistently involved in stabilizing the acarbose molecule across multiple proteins, suggesting shared or conserved binding motifs. In molecular docking, EGFR revealed the lowest binding energy of -7.8 kcal/mol. And the rest of target (ESR1, ALB, GSK3B, STAT3, AKT1, HSP90AA1, PPARG, TNF2 and SRC) dock score was -7.3 kcal/mol, -7.1 kcal/mol, -7.0 kcal/mol, -6.9 kcal/mol, -6.8 kcal/mol, -6.7 kcal/mol, -6.4 kcal/mol, -6.3 kcal/mol, -5.9 kcal/mol, respectively (Table 6.4). As shown in Figure, we found that the main forms of interaction include vanderwaal interactions, pi-pi interactions and hydrogen bonding. The results support the hypothesis that acarbose may act through a multi-target mechanism by directly engaging multiple signaling and metabolic proteins implicated in type 2 diabetes pathophysiology.

Table 6.4: Molecular docking analysis of acarbose with selected protein targets along with their PDB IDs.

S. No	Protein	PDB ID	Binding Energy (Kcal/mol)
1.	EGFR	3POZ	-7.8
2.	ESR1	1ERR	-7.3
3.	ALB	7VR0	-7.1
4.	GSK3B	4J1R	-7
5.	STAT3	6NUQ	-6.9
6.	AKT1	3O96	-6.8
7.	HSP90AA1	4BQG	-6.7
8.	PPARG	2Q6S	-6.4
9.	TNF2	1TNF	-6.3
10.	SRC	1O43	-5.9

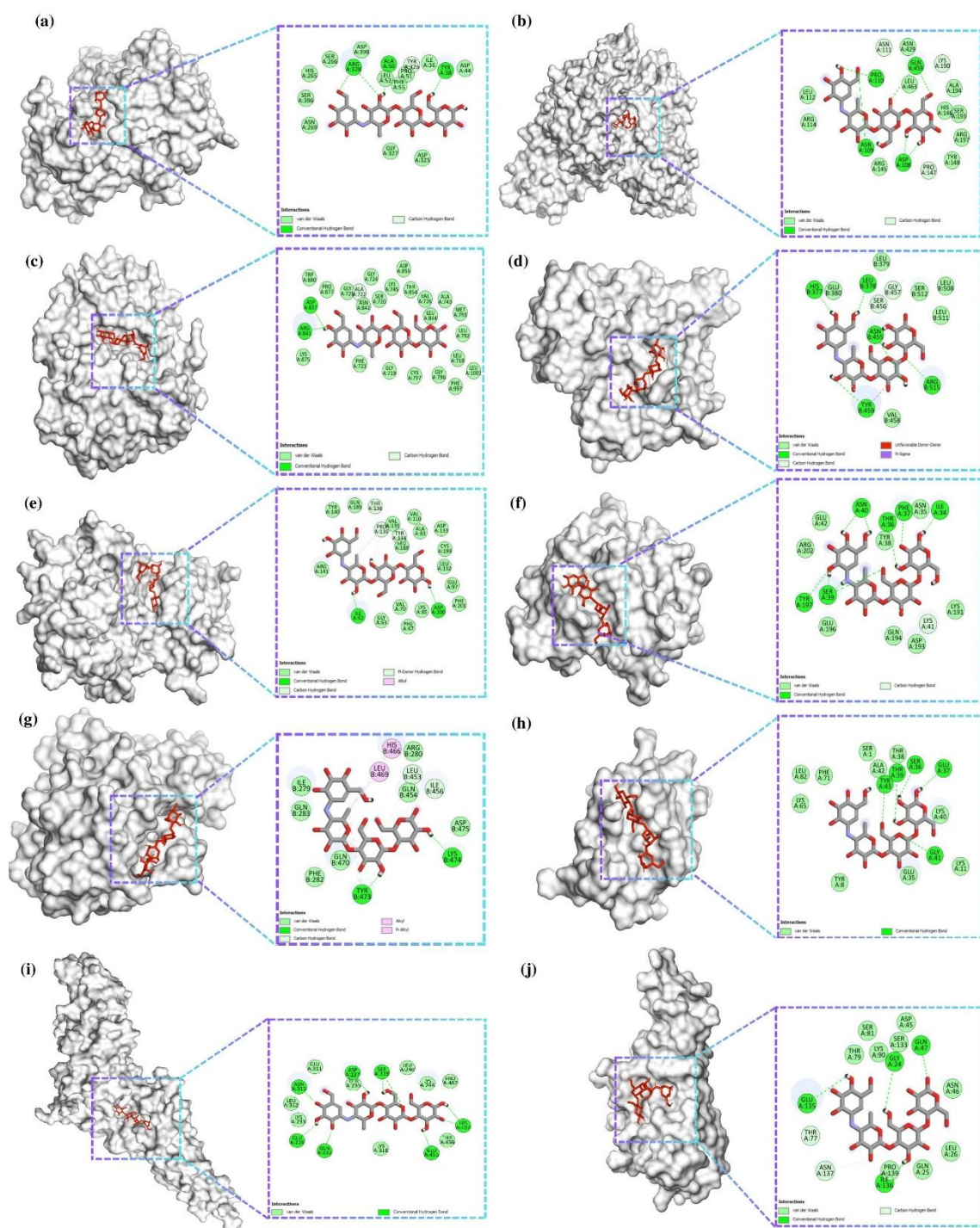
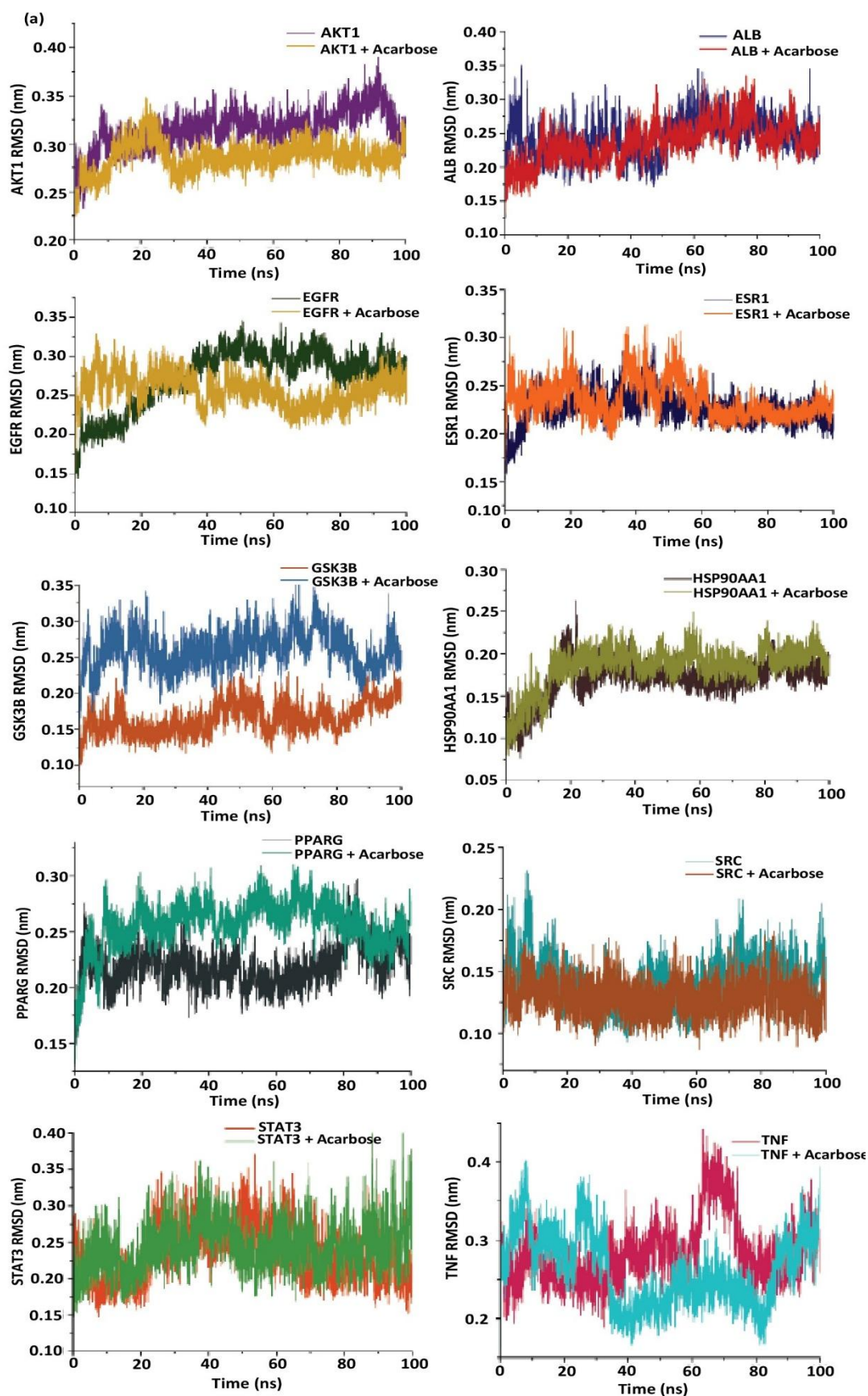


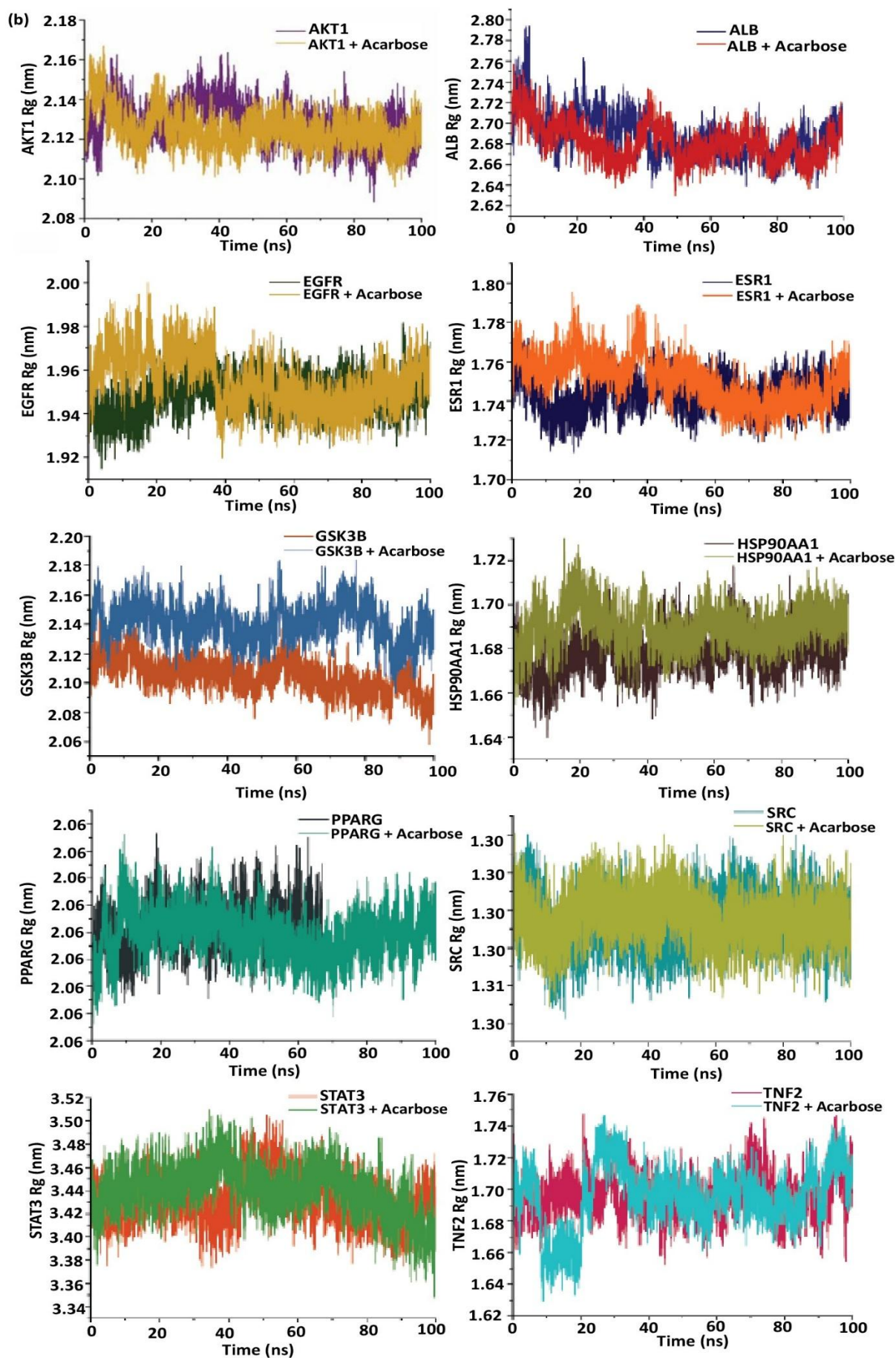
Figure 6.56: Molecular docking analysis of acarbose with the top 10 core target proteins associated with type 2 diabetes. Panels (a-j) show the docked conformations and corresponding 2D interaction diagrams of acarbose bound to each target protein. Surface representations illustrate acarbose (highlighted in red) positioned within the binding pockets of the proteins. The 2D interaction maps display hydrogen bonds, van der Waals forces, and other non-covalent interactions between acarbose and surrounding amino acid residues.

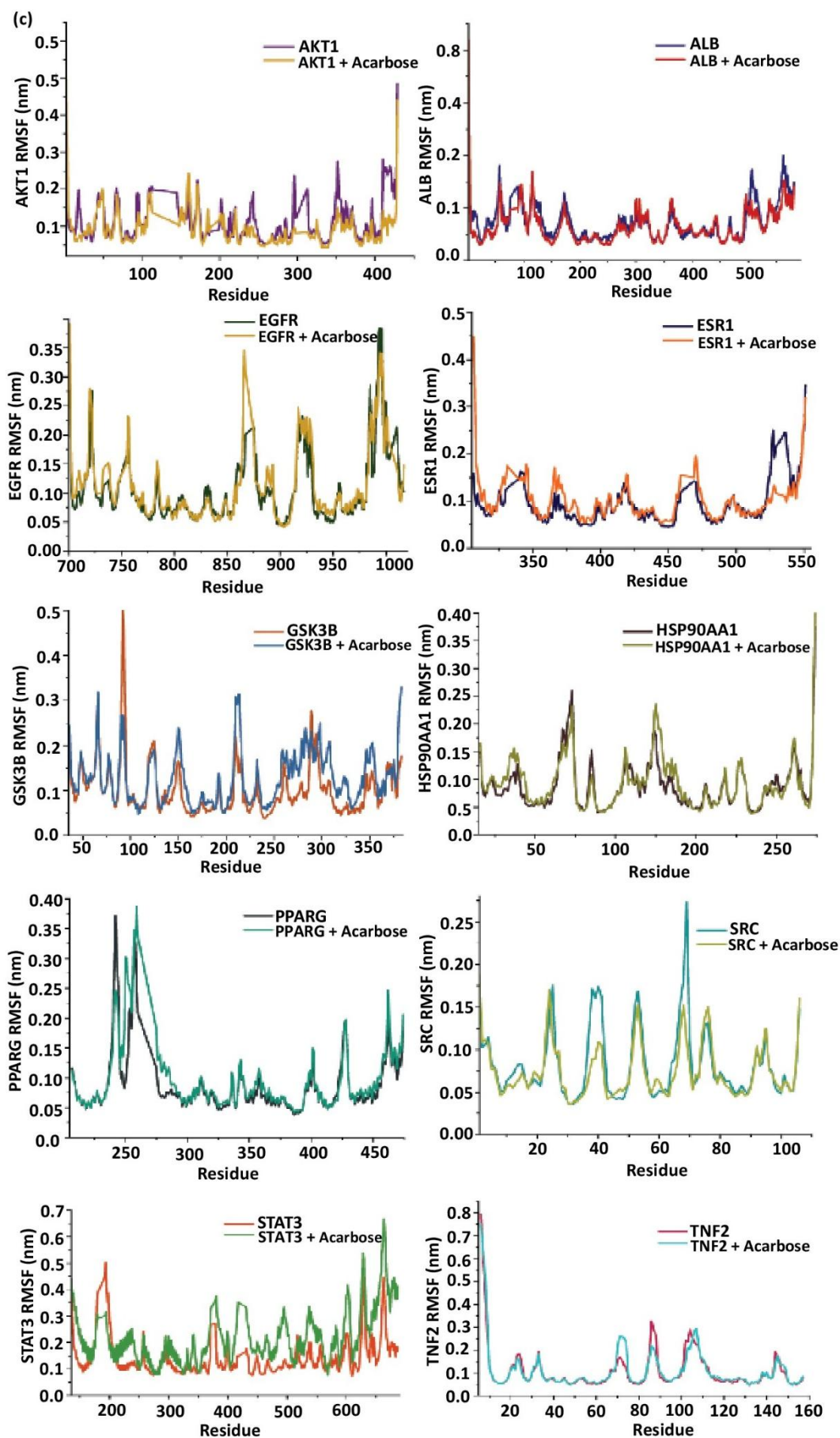
6.5.7 Molecular dynamics simulations reveal structural stability of acarbose target complexes

To evaluate the dynamic stability and structural integrity of the acarbose protein complexes, molecular dynamics (MD) simulations were conducted for the top 10 hub proteins identified in the PPI network. These included AKT1, ALB, EGFR, ESR1, GSK3B, HSP90AA1, PPARG, SRC, STAT3, and TNF. The results of the 100 ns MD simulations are presented in Figure 6.57. RMSD and RMSF plots, respectively, for both the unbound (apo) and acarbose-bound (holo) forms of the proteins. Most targets demonstrated reduced RMSD values upon acarbose binding, indicating enhanced structural stability, particularly in complexes of EGFR, STAT3, PPARG, and HSP90AA1 (Figure 6.57a-b). This stabilization suggests that acarbose binding may confer conformational rigidity to these proteins. The complexes of acarbose with PPARG and GSK3B exhibit more pronounced oscillations in their RMSD, suggesting that they are not sufficiently stable. Over the course of the simulation, the RMSDs of the other complexes steadily stabilised, suggesting that the other complex groupings stayed stable.

Figure 6.57c panel presents residue-wise RMSF plots, highlighting local flexibility differences along the protein chains. In several cases (including EGFR, TNF, PPARG), acarbose binding reduced fluctuations in active-site or functionally important regions, further supporting the notion of stable binding interactions. Radius of gyration (Rg) plots over the simulation time, providing insight into the overall compactness of each protein (Figure 6.57d). Notably, several targets, including ALB, STAT3, and GSK3B, exhibited more consistent Rg values in the presence of acarbose, suggesting a less flexible, more compact structure during the simulation. However, for all proteins, the Rg values of the protein and protein-acarbose complexes showed a similar pattern to the RMSD. As a result, all protein complexes with acarbose, with the exception of those with PPARG and GSK3B, offered exceptional dynamic structural stability while limiting the flexibility of the proteins. Collectively, these MD simulation results affirm the stable and favorable binding of acarbose to key regulatory proteins in type 2 diabetes, supporting its potential multi-target therapeutic mechanism.







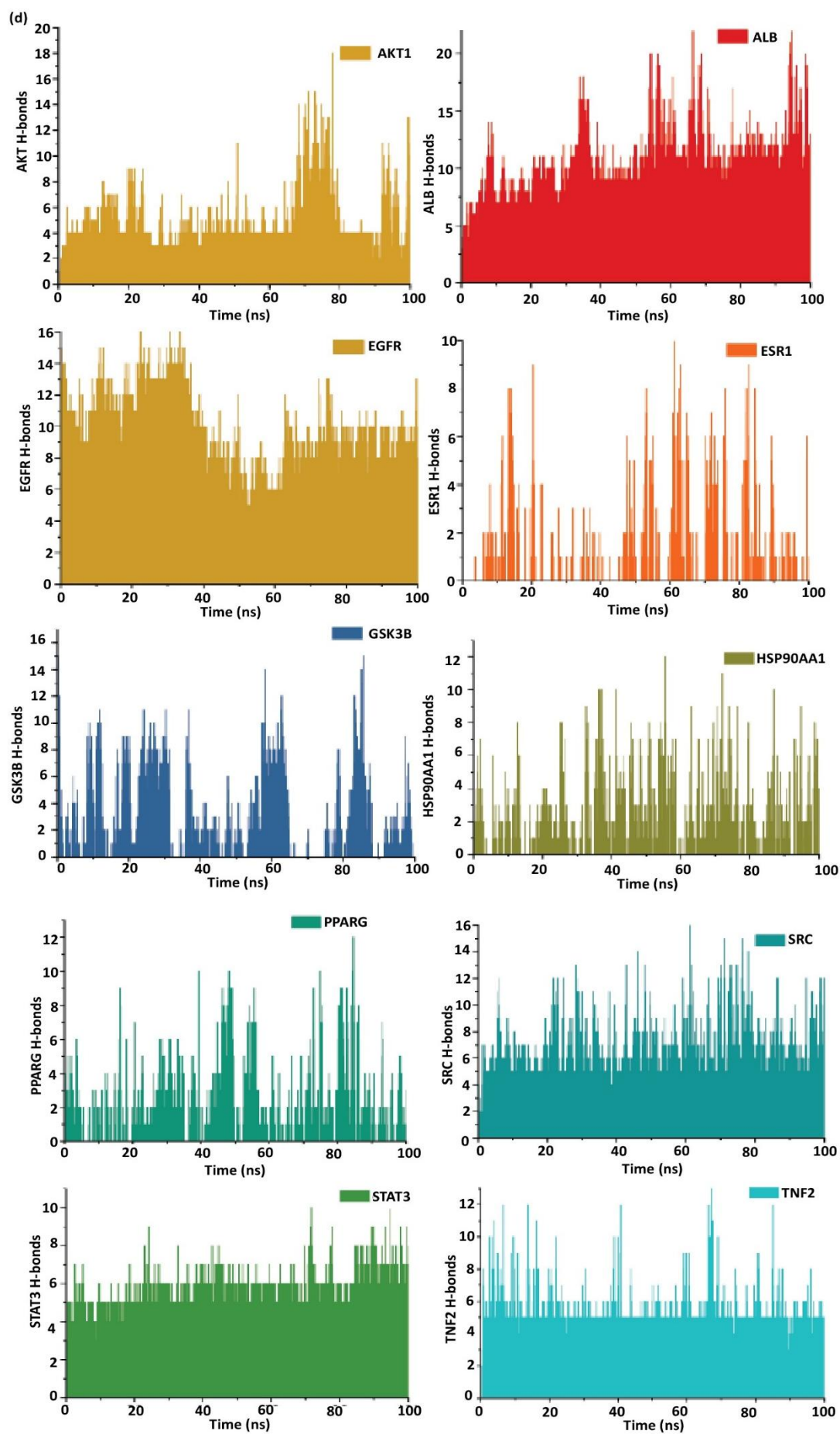


Figure 6.57: Molecular dynamics simulation analysis of Acarbose-protein complexes over a 100 ns trajectory. The dynamic behavior and stability of the 10 core Acarbose-target protein complexes were assessed using molecular dynamics (MD) simulations. **(a)** Root Mean Square Deviation (RMSD) was used to evaluate the overall structural stability of the proteins and their respective Acarbose complexes during the simulation. **(b)** Radius of Gyration (Rg) provided insights into the compactness of the protein structures in both the free and ligand-bound states. **(c)** Root Mean Square Fluctuation (RMSF) was analyzed to assess the flexibility and movement of individual residues throughout the simulation trajectory, and **(d)** The number of hydrogen bonds formed between acarbose and each target protein was tracked over time to evaluate the consistency and strength of their interactions.

Chapter 7

Discussion

A preliminary investigation was conducted to evaluate the combinational effects of acarbose with different biopolymers, including pectin, guar gum, and β -cyclodextrin. This study aimed to assess their binding affinities, antidiabetic properties, and oxidative stress modulation in *Drosophila melanogaster*. The selection of these biopolymers was based on their distinct physicochemical properties and reported biological activities that could potentially enhance therapeutic effects of acarbose. Assessing these combinations allowed for the identification of synergistic interactions that may optimize glucose regulation and mitigate oxidative stress in diabetic models.

Hyperglycemia, marked by elevated blood glucose levels, typically results from insufficient insulin or impaired cellular insulin response. This condition underlies many diabetes-related complications, including cardiovascular disease, neuropathy, nephropathy, and retinopathy (Giri et al., 2018). Managing blood glucose through lifestyle interventions like exercise and diet, or through medications, is essential to prevent these complications. When lifestyle modifications alone are insufficient, individuals may begin anti-diabetic therapy with pharmacological agents such as acarbose, which was approved by the FDA in 1995 for the management of type 2 diabetes (Nathan et al., 1993; Asif, 2014; Garg et al., 2024). Acarbose inhibits α -glucosidase, an enzyme critical to carbohydrate digestion, slowing carbohydrate absorption and reducing postprandial hyperglycemia. However, acarbose's clinical use is limited by frequent dosing needs, low oral bioavailability, and gastrointestinal side effects (DiNicolantonio et al., 2015; Wachters-Hagedoorn et al., 2007; Scheen et al., 1994; Miller et al., 2001). Additionally, increased oxidative stress, closely associated with diabetes, accelerates the progression of complications (Bhatti et al., 2022). To address these limitations, we propose combining acarbose with polymers possessing both anti-diabetic and antioxidant properties, hypothesizing that such combinations could improve glycemic control and provide added protection by reducing oxidative stress in diabetic individuals.

In view of this, computational methods were first used to predict the binding and dynamic between acarbose and its carrier polymers. Molecular docking showed favorable binding energies, with lower binding energy and strong interactions suggesting effective complex formation between acarbose and polymers. Stable RMSD values over a 400-ns simulation confirmed the acarbose-polymer complex's stability, supporting its therapeutic potential. Acarbose, a well-known α -glucosidase inhibitor, helps control postprandial blood glucose by slowing down carbohydrate digestion. In this study, acarbose showed the strongest inhibition

of both α -glucosidase and α -amylase, which agrees with earlier studies (Oboh et al., 2016; Santoso et al., 2022; Bhat et al., 2025). Pectin, guar gum, and β -CD also showed inhibitory effects individually, but when combined with acarbose (at 75:25 and 50:50 ratios), the overall inhibition was slightly reduced compared to acarbose alone. The 75:25 ratio gave better results, suggesting a concentration-dependent effect. The inhibition by pectin and guar gum is likely due to their high viscosity, gel-forming ability, and esterification, which reduce enzyme-substrate interactions by limiting substrate movement (Singh et al., 2020; Bai et al., 2021). β -CD, on the other hand, showed weak inhibition. Its cyclic structure with a hydrophilic outer surface and hydrophobic inner cavity helps in encapsulating hydrophobic molecules but doesn't interact well with the active sites of α -glucosidase or α -amylase (Wang et al., 2018). These sites require specific functional groups for hydrogen bonding or electrostatic interactions, which β -CD lacks. Therefore, combining acarbose with pectin or guar gum could enhance carbohydrate digestion inhibition through viscosity-related mechanisms and might help reduce acarbose dose and side effects. In contrast, β -CD is less suitable for this purpose.

Oxidative stress arises from an imbalance between ROS generation and the body's ability to neutralize these reactive molecules or repair the resulting damage (Garg et al., 2020; Jomova et al., 2023; Chandimali et al., 2025). Radical scavenging assays such as DPPH and ABTS are widely employed to evaluate antioxidant capacity by measuring electron or hydrogen atom donation to free radicals. In the present study, the combination of acarbose with pectin and guar gum at a 50:50 ratio significantly enhanced DPPH and ABTS radical scavenging activities, surpassing other treatment groups. Conversely, β -CD exhibited the lowest antioxidant efficacy both individually and in combination. These findings corroborate earlier reports attributing the high radical scavenging activity of pectin and guar gum to their polysaccharide structures, which possess abundant hydroxyl groups capable of donating hydrogen atoms to neutralize free radicals (Hamdani et al., 2018, 2017; Jankar et al., 2020; Zhang et al., 2013; Wikiera et al., 2021; Yan et al., 2023; Hosseini Abari et al., 2021). In contrast, β -cyclodextrin's hydroxyl groups are positioned in a manner that renders them less reactive, thus limiting its intrinsic radical scavenging ability, although it can improve antioxidant stability through inclusion complex formation (Poulson et al., 2021). Overall, these results suggest that the co-administration of acarbose with pectin and guar gum can potentiate antioxidant effects by exploiting the superior radical scavenging properties of these natural polysaccharides.

Nitric oxide (NO) plays key physiological roles but can contribute to oxidative damage under pathological conditions by forming peroxynitrite (ONOO^-) through its reaction with superoxide radicals (Beckman & Koppenol, 1996). Elevated NO levels are linked to

inflammation and chronic diseases, worsening oxidative stress (Dash et al., 2025; Martemucci et al., 2022). In this study, pectin-based formulations, especially the 50:50 pectin–acarbose combination, showed strong NO scavenging activity. This aligns with previous reports attributing the activity of pectin and guar gum to their hydroxyl and carboxyl groups that neutralize reactive nitrogen species (Dinesh et al., 2022; Ghosh et al., 2022; Koriem et al., 2014). In contrast, β -CD showed weak activity, likely because its hydroxyl groups are located on the exterior and its hydrophobic cavity limits interaction with polar radicals like NO (Hedges, 2009). Overall, combining acarbose with pectin and guar gum may enhance NO scavenging and reduce nitrosative stress.

Transition metals like iron and copper catalyze free radical formation, driving oxidative damage (Rahaman et al., 2023). Chelating agents reduce metal availability, thus limiting radical generation. In this study, pectin showed the strongest metal chelating activity, while acarbose alone had the weakest. Combining pectin with acarbose at a 50:50 ratio significantly enhanced chelation, followed by guar gum combinations. β -CD exhibited the lowest chelating capacity at the same ratio, though its combination at 25:75 improved chelation compared to other β -CD combinations. This aligns with reports that carboxyl and hydroxyl groups in pectin and guar gum drive strong metal binding (Zhaxenbay et al., 2020; Khotimchenko et al., 2012; Grant et al., 1973). These groups coordinate divalent metals per the egg-box model, underpinning their antioxidant action (Grant et al., 1973; Chandel et al., 2022; Topal et al., 2016; Hamdani et al., 2017). Since β -CD lacks carboxyl groups, it is less effective at metal chelation despite forming inclusion complexes (Sandilya et al., 2020). Thus, combining pectin or guar gum to acarbose can boost antioxidant defense via improved metal chelation, whereas the efficacy of β -CD is limited.

H₂O₂, a well-characterized ROS, plays a dual role in cellular physiology, acting as a signaling molecule at low concentrations while contributing to oxidative stress at elevated levels. Excessive H₂O₂ can give rise to highly reactive hydroxyl radicals, which disrupt the delicate balance of the cellular antioxidant defense system (Afzal et al., 2023). This disruption leads to oxidative damage of critical biomolecules, including lipids, proteins, and DNA, thereby initiating or exacerbating pathological conditions such as inflammation, neurodegenerative disorders, and cancer (Afzal et al., 2023; Jomova et al., 2023). In line with previous findings, our study demonstrated that exposure to H₂O₂ markedly reduced the activities of key antioxidant enzymes, SOD and catalase, resulting in heightened LPO and oxidative stress. Interestingly, treatment with a combination of acarbose and natural polymers, particularly guar gum and pectin in equal proportions, significantly ameliorated these effects. This combination

not only restored antioxidant enzyme activities but also reduced LPO, suggesting a protective role against H₂O₂-induced oxidative damage. Our results are supported by earlier reports that highlight the antioxidant potential of acarbose, guar gum, and pectin. These agents have been shown to mitigate oxidative stress by enhancing endogenous antioxidant defense mechanisms and reducing LPO markers (Koriem et al., 2014; Soumya et al., 2014; Liu et al., 2022). In contrast, β -CD exhibited only a minimal effect on oxidative stress parameters, underscoring the superior efficacy of the acarbose-polymer combinations tested in our study. Collectively, these findings suggest that the co-administration of acarbose with natural polymers such as guar gum and pectin may provide a synergistic antioxidant effect. This combination may serve as a promising strategy to counteract oxidative damage, particularly under conditions of elevated ROS such as H₂O₂ exposure. Future studies may further elucidate the molecular mechanisms underlying this protective effect and explore its potential therapeutic applications in oxidative stress-related disorders.

AChE hydrolyze acetylcholine to terminate synaptic transmission, and elevated AChE activity is linked to neurodegenerative diseases like Alzheimer's and Parkinson's. Xanthine oxidase, involved in purine metabolism, generates ROS, contributing to oxidative stress and conditions such as cardiovascular disease, diabetes, and renal dysfunction (Kuwabara et al., 2023). In this study, pectin and guar gum significantly inhibited AChE and Xanthine oxidase activities compared to acarbose alone, likely due to their hydroxyl and carboxyl groups interacting with enzyme active sites. H₂O₂-induced oxidative stress increased enzyme activity, but combining acarbose with pectin or guar gum, especially at a 1:1 ratio, enhanced enzyme inhibition, indicating synergistic effects. β -CD showed limited inhibition alone but additive effects when combined with acarbose, possibly by modulating drug release or stability. These inhibitory effects are comparable to standard inhibitors like galantamine and allopurinol, which bind active sites (Greenblatt et al., 1999; Šmelcerović et al., 2017). The antioxidant properties and non-toxic profiles of pectin and guar gum formulations further support their therapeutic potential (Smirnov et al., 2017; Bag et al., 2020). Overall, acarbose combined with natural polysaccharides may effectively target oxidative stress-related enzymes. Future work should focus on nanodelivery systems to improve bioavailability and efficacy.

Oxidative stress is a key driver of inflammation, with H₂O₂ playing a major role in activating inflammatory pathways. H₂O₂ promotes pro-inflammatory cytokine TNF- α production while suppressing the anti-inflammatory cytokine IL-10 (Mittal et al., 2014; Bhol et al., 2024). In this study, combining acarbose with natural polymers pectin and guar gum effectively countered H₂O₂-induced inflammation. This treatment significantly decreased TNF- α and increased IL-

10 levels in *Drosophila* larvae exposed to oxidative stress, suggesting a synergistic anti-inflammatory effect. These results support previous findings on the anti-inflammatory actions of acarbose, pectin, and guar gum across various models including population studies, *in vitro*, and *in vivo* systems (Chen et al., 2015; Li et al., 2019; Zhao et al., 2020; Zamani et al., 2023; Song et al., 2021; Van Hung et al., 2017; Jhundoo et al., 2021; Vijay et al., 2024; Ye et al., 2010; Dang et al., 2022). The dual modulation of reducing TNF- α and enhancing IL-10 highlights the therapeutic potential of combining acarbose with these natural polymers for managing chronic inflammatory conditions exacerbated by oxidative stress. Further research is needed to elucidate the molecular mechanisms and evaluate clinical relevance to fully exploit their anti-inflammatory properties.

Among the tested combinations, acarbose with pectin exhibited the most pronounced therapeutic effects, followed closely by guar gum, highlighting their potential to enhance the glucose-regulating and antioxidant properties of acarbose. Guar gum was prioritized over pectin due to its extensive cultivation in India, where it significantly contributes to the agricultural economy through exports and industrial applications. Moreover, previous studies have well-established the protective effects of guar gum in the management of diabetes, including its role in modulating glycemic response, improving lipid profiles, and exerting antioxidant and anti-inflammatory effects. These findings support the development of a guar gum-acarbose combination formulation, which may offer synergistic benefits by improving glycemic control and mitigating oxidative stress more effectively than acarbose alone.

In the present study, solid dispersion based nanoformulations of acarbose and guar gum (AEGN 1, AEGN 2, AEGN 3) were successfully developed using spray drying technology, which is widely recognized for improving the solubility and bioavailability of drugs with poor aqueous solubility and limited gastrointestinal absorption (Myślińska et al., 2023; Bhujbal et al., 2021). Spray drying promotes rapid solvent evaporation and produces dry particles with controlled morphology, particle size, and physical state, leading to enhanced dissolution rates and stability. The formulations were designed to improve key physicochemical parameters of acarbose, including stability, particle size, and surface charge. Increasing the concentration of guar gum reduced the particle size, likely due to increased viscosity of the feed solution that supports finer atomization and limits particle coalescence (Malamatari et al., 2020; Shepard et al., 2020). These findings are consistent with previous studies showing that spray drying improves both particle size and surface morphology (Guo et al., 2022; Sham et al., 2004).

Zeta potential analysis revealed that an increase in polymer content generally enhanced colloidal stability. AEGN-2 exhibited the most negative zeta potential, indicating stronger

electrostatic repulsion and better dispersion stability, which may be attributed to the ionizable hydroxyl groups present in guar gum (Öztürk et al., 2024; Ayala et al., 2013; Lesniewska et al., 2025). Although AEGN-3 contained the highest polymer content, it showed a slightly less negative zeta potential than AEGN-2. This reduction could be due to polymer entanglement or surface saturation effects, which may limit the exposure of charged functional groups on the nanoparticle surface (Ribeiro et al., 2024; Garg et al., 2023; Šeremet et al., 2024). These observations support earlier reports that increased polymer encapsulation enhances nanoparticle stability and prevents aggregation (Paul et al., 2024). Overall, spray drying improved the particle size, surface charge, and surface characteristics of acarbose guar gum formulations. The nanoscale size and stable surface potential are expected to enhance mucosal adhesion, intestinal absorption, and systemic availability of acarbose, improving its therapeutic action in diabetes. These findings contribute to existing literature on the role of polymer concentration and spray drying parameters in modifying nanoparticle-based drug delivery systems (Almansour et al., 2022; Arpagaus et al., 2022; Sharma et al., 2016; Liu et al., 2024; Jia, 2005).

SEM analysis revealed significant morphological changes resulting from the spray drying process and confirmed successful encapsulation of acarbose in the guar gum matrix. Raw acarbose exhibited a dense crystalline plate-like morphology with smooth surfaces, consistent with its highly ordered structure and poor aqueous solubility (Dilip and Joysa 2020). In contrast, guar gum showed a fibrous and irregular morphology, reflecting its semi crystalline nature and strong water binding capacity (Gunti Muthaiah et al 2019; Qiu et al 2018; Hanmantrao et al 2022; Khan et al 2024). SEM images of the spray dried nanoformulations (AEGN-1, AEGN-2, and AEGN-3) showed a clear shift to spherical or near spherical particles, typical of spray dried powders (Chopde et al 2020). This transformation is attributed to rapid solvent evaporation during atomization, which promotes solute precipitation and solidification. The observed morphology suggests efficient encapsulation and reduced crystallinity of acarbose. No distinct crystalline features of acarbose were visible in the AEGN formulations, indicating its molecular dispersion or conversion to an amorphous state within the polymer matrix. This is critical, as amorphization disrupts the crystalline lattice, enhancing solubility and dissolution. These findings support earlier reports showing that guar gum enhances encapsulation efficiency and structural stability of hydrophilic drugs (Elzayat et al 2023). The results are consistent with previous studies showing that spray drying improves the physical state of poorly soluble drugs and promotes the formation of amorphous dispersions with superior dissolution and therapeutic potential (Wilson et al 2020; Justen et al 2024; Deac et al

2025; Chuah et al 2014). In conclusion, SEM analysis confirmed that spray drying and guar gum encapsulation altered particle morphology, promoted molecular dispersion of acarbose, and improved physicochemical properties essential for effective drug delivery.

FTIR analysis revealed essential molecular-level interactions between acarbose and guar gum in the spray dried nanoformulations AEGN-1, AEGN-2, and AEGN-3. The spectrum of pure acarbose showed characteristic peaks attributed to its multiple functional groups, reflecting its crystalline structure. Guar gum exhibited spectral features typical of polysaccharides, confirming its structural profile as previously described in literature (Mawlankar et al 2020; Dilip and Joysa 2020; Mudgil et al 2012; Motta et al 2019). In the AEGN formulations, noticeable spectral changes were observed, including peak broadening and minor shifts. These changes suggest the presence of physical interactions such as hydrogen bonding between acarbose and guar gum. These interactions are consistent with known mechanisms in polysaccharide-based formulations, where hydrogen bonding facilitates drug incorporation without causing chemical degradation or covalent modification (Tran et al 2020; Puncochova et al 2014; Mustafa et al 2022).

The absence of any new peaks or loss of key spectral features confirms that the primary chemical structures of both components remained stable during spray drying. This supports the conclusion that encapsulation was achieved through non covalent interactions while preserving chemical integrity, in agreement with prior reports on spray dried polysaccharide systems (Panda et al 2016; Beck Broichsitter et al 2012; Wan and Yang 2016). These findings indicate successful molecular dispersion of acarbose within the guar gum matrix, which may enhance solubility and protect the drug from degradation. Similar results have been reported in other polysaccharide based nanoformulations that improve physical stability and prevent drug recrystallization (Pasieczna-Patkowska et al 2025; Elzayat et al 2023). Overall, the FTIR results confirm effective encapsulation driven by hydrogen bonding and physical interactions, supporting the use of guar gum for enhancing the performance of acarbose.

pXRD analysis confirmed a significant alteration in the physical state of acarbose after encapsulation in AEGN formulations. Pure acarbose showed intense and sharp diffraction peaks, indicating its crystalline nature. Guar gum displayed a broad and low-intensity peak, consistent with its semi crystalline profile, as reported earlier (Dhumal et al 2019; Abdel Raouf et al 2018; Pandey and Ramontja 2016). AEGN formulations exhibited diffuse halos in place of distinct crystalline peaks, suggesting amorphization of acarbose. This transition is attributed to rapid solvent evaporation and molecular dispersion during spray drying. Amorphous forms generally show higher energy states and better solubility due to disrupted lattice structure.

These observations are consistent with literature that emphasizes the role of spray drying in achieving amorphous drug dispersions with enhanced dissolution and bioavailability (Nyamba et al 2024; Zhang et al 2023).

In contrast, physical mixtures retained their original crystalline profiles with only slight peak broadening, likely due to mechanical mixing. This confirms that simple blending does not induce amorphization. These observations are consistent with literature that emphasizes the requirement of thermomechanical or solvent driven processes for effective solid-state modification (Saha et al 2023; Kim et al 2021; Bhalani et al 2022). Moreover, guar gum played a dual role by not only facilitating dispersion but also stabilizing the amorphous form. Its polymeric network likely restricted molecular mobility and minimized recrystallization. This stabilization contributes to the improved encapsulation and uniformity seen in AEGN formulations, supporting the application of polysaccharide-based matrices for enhancing drug solubility and performance.

The assessment of drug content and drug entrapment efficiency in the AEGN nanoformulations demonstrated a clear correlation between increased polymer concentration and improved encapsulation outcomes. This trend is consistent with prior reports that emphasize the fundamental role of polymer content in enhancing drug entrapment during nanoparticle formulation. As polymer concentration increases, the resulting denser and more cohesive polymeric matrix facilitates more efficient incorporation of drug molecules, thereby reducing the likelihood of drug loss during processing (Özdal et al., 2022; Venkidasamy et al., 2025; Zafar et al., 2023). This phenomenon is attributed to the ability of polymers to form a robust network that stabilizes the drug within the nanoparticle core, leading to increased structural integrity and reduced surface-associated drug leakage. Several studies have highlighted that a higher polymer-to-drug ratio enhances the mechanical strength and stability of nanoparticles, supporting sustained drug retention and preventing premature release (Haider et al., 2022; Floyd et al., 2025; Lee et al., 2021; Rojas et al., 2024). Moreover, the improved encapsulation efficiency observed at elevated polymer concentrations contributes to a more controlled and prolonged drug release profile, which is desirable for maintaining therapeutic plasma levels over extended durations. This not only improves drug bioavailability but also minimizes dosing frequency and associated side effects. Overall, the findings in AEGN formulations reaffirm the pivotal role of polymer concentration in modulating encapsulation efficiency. By enhancing drug stability and promoting sustained delivery, polymer-rich formulations offer a strategic advantage in the development of effective nano-based therapeutic systems.

Importantly, under reduced hydration conditions, the compatibility of the formulation may be affected due to limited polymer swelling and restricted hydrogen bonding interactions between guar gum and acarbose. Since hydration drives matrix expansion and stabilization of the amorphous drug state, reduced water availability could weaken molecular dispersion and increase the risk of partial recrystallization. This may, in turn, alter drug-polymer interactions observed in FTIR and XRD studies, potentially compromising encapsulation stability and uniform release. However, the strong non-covalent interactions and amorphous stabilization provided by guar gum in AEGN formulations suggest that even under suboptimal hydration, basic compatibility is retained, though performance efficiency could be moderately reduced. The spray-dried acarbose-guar gum nanoformulations demonstrated robust physicochemical stability during a six-month accelerated stability study conducted in accordance with ICH Q1A(R2) guidelines. When stored under stressed conditions ($40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ RH), no significant alterations were observed in particle size, PDI, zeta potential, entrapment efficiency, drug content, or *in vitro* release profiles, indicating preservation of structural integrity and drug-retaining capacity. The observed stability can be attributed to the structural robustness of the galactomannan backbone of guar gum, which has been reported to withstand moderately elevated temperature and humidity stresses while maintaining viscosity within $25\text{--}40^\circ\text{C}$ (Tahmouzi et al., 2023; Mudgil et al., 2016). These findings are consistent with previous reports on guar gum-based delivery systems, highlighting its suitability as a stabilizing polymer in oral formulations (Kothari et al., 2015).

In vitro drug release studies performed at pH 6.8, representing intestinal conditions, revealed sustained release of acarbose, confirming effective encapsulation and controlled delivery. Although release studies under gastric pH (1.2) and hydration-dependent swelling analyses were not performed in this study, literature suggests that guar gum exhibits minimal swelling and limited drug release under acidic conditions, while swelling and controlled release are enhanced in neutral to slightly basic environments, consistent with its polysaccharide gel-forming properties (Ashames et al., 2022; Das & Subuddhi, 2015; Seeli et al., 2016; Mudgil et al., 2014). The maintenance of encapsulation efficiency and sustained drug release throughout the study indicates that acarbose-guar gum interactions effectively mitigate premature drug leakage or degradation. Comparable protective effects of drug-polymer interactions have been reported in other polysaccharide-based nanoformulations, where interpolymer interactions enhance stability and controlled release behavior (Bianchera & Bettini, 2020; Garg & Gupta, 2023). While accelerated stability testing provides predictive insights, real-time studies under varying packaging, humidity, and long-term storage conditions remain necessary for

comprehensive shelf-life validation. Collectively, these results support the efficacy of guar gum as a stabilizing and sustained-release polymer in spray-dried acarbose nanoformulations, with potential application in commercial oral antidiabetic delivery systems.

The antioxidant potential of the developed nanoformulations was systematically evaluated using a range of radical scavenging assays, including DPPH, ABTS, metal chelation, and nitric oxide scavenging assays. Standard reference antioxidants such as L-ascorbic acid, Trolox, EDTA, and Vitamin C were employed for comparative analysis. These assays are widely used to assess the ability of compounds to neutralize ROS and other free radicals, which play a significant role in the pathophysiology of oxidative stress-related diseases, particularly diabetes. Our findings revealed that the acarbose-guar gum nanoformulations exhibited considerable free radical scavenging and metal chelation activity, suggesting a strong potential to mitigate oxidative damage. This aligns with previous reports demonstrating the antioxidant capabilities of both acarbose and guar gum, individually and in combination with other bioactive compounds (Garg et al., 2025; Soumya et al., 2014; Oboh et al., 2016; Hamdani et al., 2017, 2018; Marouf & Ghorbani, 2022; Pu et al., 2018; Jiang et al., 2022; Wang et al., 2025; Dodi et al., 2023). Acarbose, although primarily known for its α -glucosidase inhibitory action, has also been reported to modulate redox homeostasis and exert antioxidant effects by scavenging ROS (Oboh et al., 2016). This dual functionality enhances its therapeutic relevance, especially in conditions like diabetes where oxidative stress exacerbates disease progression. When combined with naturally derived polymers such as guar gum, the antioxidant efficacy appears to be further potentiated. Guar gum contains multiple hydroxyl groups capable of donating hydrogen atoms to neutralize free radicals and chelate transition metal ions, thus contributing to its antioxidant properties (Hamdani et al., 2017; Wagner et al., 2008). The observed synergistic antioxidant activity in our formulations can likely be attributed to the combined effects of acarbose and the hydroxyl-rich guar gum matrix. This enhanced radical scavenging and metal chelation capacity support the potential utility of these nanoformulations in counteracting oxidative stress commonly observed in diabetic conditions.

Management of postprandial glucose is critical in type 2 diabetes. This relies on modulating carbohydrate digestion, which involves α -amylase and α -glucosidase activities that accelerate glucose release from polysaccharides (DiNicolantonio et al 2015; Aguerd et al 2025; Dirir et al 2022; Guo et al 2021). Inhibiting these enzymes delays glucose absorption and improves glycemic control. Acarbose is a known inhibitor of both enzymes, limiting carbohydrate breakdown in the intestine. Guar gum, a soluble fiber, complements this action through viscosity enhancement, which slows glucose diffusion and promotes satiety. Studies report that

guar gum potentiates acarbose mediated α -glucosidase inhibition, likely by extending drug contact time with enzymes (Garg et al 2025). This synergistic effect is further supported by reports on guar gum mediated enhancement of enzyme sensing using gold nanoclusters (Geng et al 2023). The present findings align with these observations, as AEGNs demonstrated superior inhibition, likely due to combined actions of acarbose and guar gum. Acarbose interferes with enzymatic active sites (Dirir et al 2022), while guar gum delays glucose transit and reduces absorption rate (Hamdani et al 2018). The hydroxyl rich structure of guar gum may also support stronger enzyme interaction, enhancing its inhibitory potential (Oboh et al 2016; Garg et al 2025). The nanoformulation possibly improved bioavailability and prolonged the action of both agents, resulting in enhanced efficacy (Garg et al 2025; Jiang et al 2022). These findings are consistent with literature that emphasizes the therapeutic benefit of integrating enzyme inhibitors with natural polymers for glycemic regulation (Soumya et al 2014).

In vitro cytotoxicity assessment remains a critical step to ensure the safety of therapeutic agents prior to *in vivo* evaluation. This approach identifies potential toxic effects on normal cells, promoting selection of biocompatible candidates and supporting ethical research by minimizing unnecessary animal use (Buranaamnuay, 2021). In this study, AEGN formulations, acarbose, and guar gum showed favourable biocompatibility with cell viability exceeding 60% across tested concentrations. These findings align with prior reports confirming the safety of natural polysaccharides like guar gum and acarbose in non-cancerous models (Le et al., 2022; Chuang et al., 2020). Similarly, minimal toxicity was reported for biodegradable polymer-based delivery systems (Bhubhanil et al., 2021), consistent with our results. Notably, AEGN formulations, especially AEGN-3, maintained high biocompatibility in metabolically relevant cells (Caco-2 and HepG2), with only slight reduction in viability at higher concentrations, without indicating harmful effects. This observation underscores the safety of these formulations for anti-diabetic applications, rather than implying anticancer activity.

Enhanced uptake and retention of the formulations in these cell lines may contribute to the slight reduction in viability observed at higher concentrations, influenced by cell-specific endocytosis and metabolic activity, while remaining safe at therapeutically relevant doses. Conversely, acarbose and guar gum alone showed minimal cytotoxic effects, which aligns with their primary function as anti-hyperglycemic agents rather than cytotoxic compounds (Chuang et al., 2020). Collectively, these observations endorse the biocompatibility and therapeutic potential of AEGN formulations, notably AEGN-3, for anti-diabetic applications. This safety

profile supports the use of natural polymer-based nanocarriers to enhance drug efficacy while minimizing off-target toxicity. These findings support observations from Bhubhanil et al. (2021), Zarbab et al. (2023), and Dodi et al. (2023), which highlight biopolymer nanocarriers as enhancers of therapeutic outcomes with reduced off-target toxicity. Furthermore, these results align with delivery strategies discussed by Das et al. (2020) and the biocompatibility profiles of composite scaffolds described by Caldera-Villalobos et al. (2022), reinforcing the clinical promise of natural polymer-based nanoformulations. Overall, the data justify further *in vivo* studies to confirm safety and efficacy of these AEGN formulations.

LDH release is a well-established indicator for assessing cell membrane integrity and cytotoxicity. As a cytoplasmic enzyme, LDH is released into the extracellular environment when the plasma membrane is compromised, making it a reliable marker of cellular damage (Kumar et al., 2018; Kaja et al., 2017; Lin et al., 2018). The LDH assay is valuable in early-stage *in vitro* testing as it provides insights into biocompatibility prior to *in vivo* validation. Its quantitative and non-invasive nature makes it an essential tool for evaluating membrane stability and predicting toxicity (Gupta, 2022; Thomas et al., 2016). In this study, cytotoxic potential of acarbose, guar gum, and AEGN formulations was evaluated in HepG2 and L6 cell lines using LDH release assays. Acarbose induced increased LDH release in a concentration-dependent manner, indicating membrane disruption, possibly related to oxidative stress from its α -glucosidase inhibitory activity (Chuang et al., 2022). Guar gum demonstrated a more favorable profile with limited membrane damage, supporting its known biocompatibility and membrane-stabilizing properties typical of natural polysaccharides (Le et al., 2022). Among the AEGN formulations, AEGN-3 showed the lowest LDH release, reflecting enhanced biocompatibility likely due to its optimized polymer composition that mitigates membrane damage and oxidative stress. These results align with previous studies highlighting cytoprotective effects of biopolymer-based nanocarriers (Bhubhanil et al., 2021; Hanmantrao et al., 2022).

AEGN-1 and AEGN-2 showed moderate membrane disruption, suggesting relative safety but inferior to AEGN-3. In L6 cells, acarbose caused greater membrane damage compared to HepG2 cells, consistent with reports linking acarbose to mitochondrial dysfunction and oxidative stress in skeletal muscle (Chuang et al., 2020). Guar gum again maintained its protective effect in muscle cells (Le et al., 2022). AEGN-3 demonstrated superior cytocompatibility compared to acarbose, preserving membrane integrity across cell types. This improved safety profile, combined with its previously observed selective cytotoxicity toward

cancer cells, highlights the dual therapeutic potential of AEGN-3. The protective effect of guar gum contributes to overall safety, but the nanoformulated structure of AEGN-3 further enhances biocompatibility by optimizing drug delivery, reducing direct cytotoxicity, and likely facilitating controlled release that mitigates oxidative membrane damage. These findings are consistent with literature supporting the use of natural polymer-based nanoformulations to enhance therapeutic efficacy while minimizing off-target effects (Bhubhanil et al., 2021; Zarbab et al., 2023; Dodi et al., 2023). Collectively, these data support the promise of AEGN-3 as a safe and effective drug delivery system, warranting further investigation *in vivo*. This study reinforces the importance of LDH assays in early drug screening and contributes to the growing evidence for natural polymer-based nanoformulations in drug development.

Chronic hyperglycemia, a hallmark of diabetes, is a critical driver of oxidative stress through excessive production of ROS such as superoxide anion, hydrogen peroxide, and hydroxyl radicals (An et al., 2023; González et al., 2023; Rotariu et al., 2022; Singh et al., 2011; Volpe et al., 2018). The mechanisms contributing to hyperglycemia-induced oxidative stress involve both enzymatic and non-enzymatic pathways. Glucose autooxidation directly generates ROS via oxidation of glucose molecules (Choi et al., 2008; Rolo and Palmeira, 2006). Furthermore, excess glucose leads to the formation of AGEs through non enzymatic glycation of biomolecules. Interaction of AGEs with RAGE activates pro inflammatory signaling pathways including nuclear factor kappa B (NF- κ B), amplifying ROS production and inflammatory responses (Garg et al., 2020). Additionally, mitochondrial dysfunction induced by glucose overload exacerbates ROS formation by disrupting the electron transport chain, further impairing redox balance (Ceriello et al., 2016; Ighodaro, 2018). In line with this, our study demonstrated a significant increase in LPO, a reliable marker of lipid oxidative damage, under HG conditions. This supports established evidence that ROS attack polyunsaturated fatty acids in cell membranes, resulting in lipid hydroperoxides and reactive aldehydes like MDA that compromise membrane integrity (Nicolson and Ash, 2014; Olufunmilayo et al., 2023). Such oxidative damage disrupts membrane bound enzymes and cellular functions, contributing to diabetic complications (Busik et al., 2008; de Souza Bastos et al., 2016; Gray et al., 2024; Habib et al., 1994). Correspondingly, antioxidant enzymes SOD, catalase, and GST were markedly suppressed in HG treated cells, indicating impaired cellular defense against ROS (Guo et al., 2022; Jamal Gilani et al., 2021; Tekin and Seven, 2022). SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, which is then detoxified by catalase and GST; thus, reduction of these enzymes disrupts redox homeostasis.

Treatment with acarbose and guar gum partially reversed oxidative stress induced by hyperglycemia. Acarbose, an α -glucosidase inhibitor, modestly improved antioxidant status likely by reducing postprandial glucose spikes and consequent ROS formation (Chuang et al., 2020; Garg et al., 2025). Guar gum, a natural polysaccharide with inherent antioxidant properties, exhibited greater efficacy in restoring antioxidant enzyme activities and lowering LPO. Its polyphenol-rich composition is known to scavenge free radicals and restore redox balance, consistent with reports on the protective role of natural polysaccharides against oxidative damage (Kuo et al., 2009; Le et al., 2022; Soumya et al., 2014; Zhao et al., 2024). Among tested formulations, AEGN-3 demonstrated the most potent antioxidant effects, significantly reducing LPO and restoring activities of SOD, catalase, and GST close to normal levels. This superior efficacy may arise from synergistic interactions of bioactive compounds encapsulated within AEGN-3, enabling controlled release, enhanced cellular uptake, and sustained antioxidant activity. The nanoformulated structure likely facilitates improved bioavailability and effective ROS scavenging, supporting redox homeostasis restoration. These results align with previous findings on polymer-based delivery systems that augment antioxidant defenses and prolong therapeutic effects (Bhubhanil et al., 2021; Soumya et al., 2014). Overall, these findings confirm that high glucose promotes oxidative stress through increased ROS generation and impaired antioxidant mechanisms. The natural polymer based nanoformulation AEGN-3 effectively mitigates this oxidative damage, highlighting its promise as a therapeutic strategy to prevent or reduce diabetic complications caused by hyperglycemia induced oxidative stress.

The hemotoxicity assay results showed that AEGN-1, AEGN-2, and AEGN-3 induced minimal hemolytic activity at all tested concentrations, remaining well below the non hemolytic threshold of ten percent. These outcomes are consistent with prior reports demonstrating the hemocompatibility of nanoformulations designed for oral and intravenous administration (Mehta et al., 2025; Amin et al., 2006; Pathania et al., 2023; Tkachenko, 2024). Although acarbose exhibited slightly higher hemolytic activity compared to the AEGN formulations, it stayed within the safe non hemolytic range, confirming its general hemocompatibility. The improved hemocompatibility of AEGNs relative to acarbose alone likely results from nanoparticle encapsulation, which acts as a protective barrier reducing direct interaction between active agents and erythrocyte membranes. This encapsulation reduces surface charge interactions and prevents membrane destabilization, thereby limiting hemolysis (Yedgar et al., 2022). Furthermore, the polymer matrix in AEGNs facilitates controlled drug release, which

decreases acute erythrocyte exposure to active drug molecules, a common cause of hemolytic stress observed in conventional formulations. Co-administration of guar gum with acarbose further lowered hemolytic activity, emphasizing the protective role of this natural polymer. This protection is attributed to guar gum's mucoadhesive and barrier forming properties, which create a viscous shield around erythrocytes, preventing direct contact with acarbose and reducing membrane damage (Sarkar et al., 2025). Such barrier effects align with earlier studies showing that polysaccharides inhibit erythrocyte lysis by physically blocking hemolytic agents (Gopinath et al., 2018). Overall, these findings highlight the superior hemocompatibility of AEGNs compared to acarbose alone. Nanoparticle encapsulation decreases erythrocyte exposure to the drug and enhances membrane stability, while guar gum further enhances this protection, supporting the use of AEGNs as a safer therapeutic option with minimal hemotoxic risk.

All developed AEGN formulations demonstrated promising *in vitro* outcomes, including enhanced encapsulation efficiency, sustained drug release, improved anti-diabetic activity, and attenuation of hyperglycemia-induced oxidative stress in cultured cells. Consequently, their therapeutic efficacy was further assessed in an *in vivo* diabetes model. Among the formulations evaluated, AEGN-3 consistently showed superior performance in acarbose encapsulation, prolonged drug release, and biological efficacy. This formulation effectively suppressed oxidative stress under hyperglycemic conditions. Based on these results, AEGN-3 was selected for *in vivo* studies due to its superior therapeutic potential.

Persistent hyperglycemia and excessive weight gain are well-established pathological features of type 2 diabetes, typically driven by insulin resistance, β -cell dysfunction, and disordered energy metabolism (Garg and Gupta, 2022; Ruze et al., 2023). To closely mimic these conditions in animal models, the combination of HFD feeding and low-dose STZ administration is widely used due to its ability to mimic the metabolic and pathological features of type 2 diabetes (Srinivasan et al., 2005; Guo et al., 2018; Skovsø, 2014; Zhang et L., 2008). Mechanistically, HFD contributes to the development of insulin resistance and obesity by promoting excessive caloric intake, leading to adipocyte hypertrophy and visceral fat accumulation. The resulting lipotoxicity activates inflammatory pathways, including NF- κ B, JNK, and TLR4, which in turn trigger the release of pro-inflammatory cytokines such as TNF- α and IL-6 (Hardy et al., 2012; Cavaliere et al., 2023; Garg et al., 2023). These cytokines impair insulin signaling by downregulating GLUT-4-mediated glucose uptake and disrupting insulin receptor substrate activity, culminating in systemic insulin resistance. Additionally, HFD

increases circulating free fatty acids and promotes ectopic lipid deposition in hepatic and muscle tissues, further exacerbating metabolic dysfunction and resulting in significant increases in body weight and adiposity (Srinivasan et al., 2005).

STZ, a nitrosourea compound, selectively targets pancreatic β -cells via GLUT2 transporters. Once internalized, STZ induces DNA alkylation and oxidative stress, leading to β -cell apoptosis and impaired insulin secretion. When administered at a low dose following HFD feeding, STZ induces partial β -cell destruction, effectively mimicking the progressive β -cell failure observed in type 2 diabetes rather than complete insulin deficiency (Guo et al., 2018; Zhang et al., 2008). The combination of HFD-induced insulin resistance and STZ-induced β -cell impairment results in sustained hyperglycemia, glucose intolerance, dyslipidemia, and obesity, thus providing a robust and reliable preclinical model for evaluating anti-diabetic and anti-obesity therapies (Wolosowicz et al., 2022; Bouyahya et al., 2024; Singh et al., 2014; Hu et al., 2022). In the present study, HFD-fed, STZ-induced diabetic rats demonstrated significantly elevated fasting blood glucose levels and increased body weight over time, consistent with the dual pathology of insulin resistance and β -cell dysfunction (Skovsø, 2014; Magalhães et al., 2019; Tian et al., 2021; Ren et al., 2020). Numerous studies have highlighted the anti-diabetic potential of acarbose and guar gum. Acarbose, an α -glucosidase inhibitor, delays carbohydrate digestion in the intestine, thereby reducing postprandial glucose excursions. Guar gum, a soluble dietary fiber, slows glucose absorption by increasing intestinal viscosity and promoting satiety (Slavin, 2005; Cani et al., 2008; Li et al., 2023). A study by Zhao et al. showed that acarbose at a dose of 50 mg/kg for consecutive 14 days significantly lowered fasting blood glucose in STZ-induced diabetic C57BL/6 mice, suggesting a protective role in preserving β -cell function and reducing insulinitis (Zhao et al., 2020). In contrast, although acarbose reduced postprandial glucose and insulin levels in BB/Wor rats (a type 1 diabetes model), it failed to significantly reduce disease incidence or delay onset, though it helped stabilize blood glucose under high-sucrose diets (Koevary, 1990). A study involving sucrose-fed obese and lean SHR/N-corpulent rats showed that 12-week acarbose supplementation decreased body weight in obese rats, while improving glucose tolerance and insulin response, indicating its relevance in managing non-insulin-dependent diabetes mellitus (Carswell et al., 1989).

Previous research has also emphasized that dietary fibers and plant-derived polysaccharides modulate gut microbiota, delay gastric emptying, and regulate appetite-related hormones, thereby reducing caloric intake and improving metabolic profiles (Slavin, 2005; Cani et al.,

2008). For example, in human subjects, a low dose of guar gum (2.5 g) significantly reduced postprandial glucose levels (Torsdottir et al., 1989). Similarly, meals containing soluble fibers (guar gum, xanthan gum, methylcellulose) lowered postprandial glucose levels more than insoluble fibers like wheat bran (Cameron-Smith et al., 1994). In diabetic rats, guar gum (20% w/w) reduced blood glucose and body weight (Frias and Sgarbieri, 1998), and significantly suppressed food intake and weight gain (Frias and Sgarbieri, 1998). Furthermore, graded guar gum diets (0%, 5%, 10%, 20% w/w) in STZ-induced diabetic rats over 28 days resulted in a dose-dependent decrease in blood glucose and body weight (Samarghandian et al., 2012).

In the present study, treatment with acarbose and guar gum significantly reduced fasting blood glucose levels and body weight in HFD-fed, STZ-induced diabetic rats, corroborating previous findings that support their efficacy in mitigating hyperglycemia and obesity-related outcomes. Notably, among the developed nanoformulations, AEGN-3 administered at a dose of 50 mg/kg demonstrated the most pronounced reduction in both fasting glucose and body weight compared to treatment with acarbose or guar gum alone. This enhanced therapeutic effect may be attributed to the synergistic action of multiple mechanisms, including potent inhibition of α -glucosidase, improved peripheral insulin sensitivity, and attenuation of oxidative stress. Although guar gum has been reported to reduce postprandial glycemic excursions due to its soluble fiber content, its effect was comparatively modest. The superior efficacy observed with AEGN-3 likely stems from its optimized physicochemical properties, such as higher encapsulation efficiency, sustained drug release kinetics, and enhanced cellular uptake of the bioactive agents, collectively contributing to a more robust and prolonged antihyperglycemic response.

Diarrhoea is a common gastrointestinal side effect associated with disrupted water absorption, increased motility, or osmotic imbalances. Acarbose, an α -glucosidase inhibitor, delays carbohydrate digestion in the small intestine, allowing undigested carbohydrates to reach the colon where bacterial fermentation produces osmotically active byproducts such as short-chain fatty acids and gases, increasing luminal osmolarity and accelerating intestinal transit (Lettieri and Dain, 1998). Although flatulence is more commonly reported, diarrhoea remains a notable adverse effect contributing to poor compliance. For instance, 13.7% of patients reported flatulence and 2.2% experienced diarrhoea in a clinical study, while 83% tolerated acarbose without any adverse effects (Spengler et al., 2005). While another study showed that rats treated with an acarbose–extract combination showed a significantly lower inhibition of stool weight up to 53.80%, compared to other experimental groups, emphasizing the role of acarbose in

contributing gastrointestinal side effects (Ohadoma et al., 2020). In agreement to these findings, our study showed that acarbose at a dose of 50 mg/kg induced marked diarrhoea in rats, with peak scores of 3 by Day 19, which tapered to 1-2, likely due to microbial adaptation (Hanefeld, 2007).

Conversely, guar gum has been known to demonstrate beneficial gastrointestinal effects due to its galactomannan-based soluble fiber composition, which increases luminal viscosity, slows glucose absorption, and enhances water-binding properties. The supplementation of 2% guar gum-enriched diet showed a notable reduction in diarrhoea, compare to those who are on fibre-free diet (Rushdi et al., 2004). Likewise, pelvic radiation patients showed reduction in diarrhoea upon receiving guar gum at 20 g/day for consecutive 28 days (Rosli et al., 2022). Our study corroborates these findings by showing that rats treated with guar gum at a dose of 200 mg/kg for 21 days experienced only transient, mild elevations in diarrhoea scores, confirming its gastrointestinal safety. Notably, AEGN-3 at both 25 and 50 mg/kg showed minimal gastrointestinal effects, suggesting enhanced tolerability compared to acarbose. This reduction may be attributed to the ability of guar gum to increase luminal viscosity and reduce abrupt carbohydrate malabsorption and fermentation. Overall, our findings position AEGN-3 as a promising antidiabetic agent with minimal gastrointestinal side effects.

The present study demonstrates the significant impact of chronic hyperglycemia on hepatic function in the HFD-fed, STZ-induced diabetic rat model, widely used to mimic type 2 diabetes and related metabolic disturbances including liver dysfunction. In this study, HFD-fed, STZ-induced animals exhibited significant liver damage, as evidenced by elevated hepatic biomarkers such as bilirubin, SGOT, SGPT, ALP, GGT, and LDH, corroborating previous findings that HFD aggravates hepatic injury by inducing insulin resistance, lipid accumulation, and ER stress. These pathological changes disrupt liver architecture and function, as reflected by increased enzyme leakage and elevated hepatic injury markers (Kalas et al., 2021; Mohamed et al., 2016; Bi, 2024; Ahmadiéh and Azar, 2014; Bae et al., 2023; Mobasheri et al., 2023). Treatment with acarbose significantly decreased liver enzymes, especially SGPT and SGOT, supporting its hepatoprotective role and improvement in liver histology (Husen et al., 2020). Similarly, guar gum, particularly its partially hydrolyzed form, has been reported to protect the liver against toxic insults by lowering serum LDH, AST, and ALT (Wu et al., 2019). Both agents individually improved liver biochemical parameters in diabetic rats in this study. Notably, AEGN-3 treatment exhibited superior efficacy in restoring hepatic function, significantly reducing bilirubin, SGOT, SGPT, ALP, GGT, and LDH compared to diabetic controls. This

enhanced effect likely results from improved bioavailability and synergistic antioxidant and anti-inflammatory actions, neutralizing ROS, suppressing inflammatory mediators, and stabilizing hepatocyte membranes. These findings highlight AEGN-3 as a promising hepatoprotective agent in diabetes by targeting oxidative stress, inflammation, and metabolic dysfunction. Future studies involving histological evaluation and molecular pathway analyses are warranted to further elucidate AEGN-3's protective mechanisms and clinical potential.

Diabetic nephropathy remains a common and severe complication of chronic hyperglycemia, particularly in experimental models induced by STZ and HFD. Persistent hyperglycemia causes progressive renal dysfunction primarily through oxidative stress and inflammation. Excessive ROS production damages glomerular and tubular structures, impairing filtration and renal clearance, as evidenced by elevated serum creatinine, urea, and uric acid levels, key markers of renal injury (Guo et al., 2021; Shen et al., 2023; Ni et al., 2019). Hyperglycemia also stimulates pro-inflammatory cytokines including TNF- α , IL-6, and IL-1 β , and leads to AGE accumulation which interacts with RAGE on renal cells to activate inflammatory and fibrotic signaling pathways. These processes result in glomerulosclerosis, mesangial expansion, and further renal damage (Wendt et al., 2003; Fakhruddin et al., 2017). Consistent with these mechanisms, HFD-fed, STZ-induced diabetic rats in this study showed significant increases in serum urea, creatinine, and uric acid, reflecting impaired renal function. Acarbose, an α -glucosidase inhibitor that controls postprandial glucose, has demonstrated renoprotective effects by reducing glucotoxicity and oxidative stress. Studies in HFD-fed db/db mice reported acarbose therapy lowered nephrotoxicity markers through suppression of Ras-mediated pathways (Hung et al., 2022). Similarly, long-term acarbose treatment improved renal morphology and function in alloxan diabetic rats (Macedo et al., 1996). In agreement, acarbose administration (50 mg/kg) in this study significantly reduced elevated renal biomarkers, supporting its nephroprotective role. Guar gum, a soluble fiber with antioxidant and anti-inflammatory properties, also showed significant renoprotection. Prior reports documented its efficacy in reducing nephrotoxicity markers in mercury-exposed rats (Abd El-Rhman and Shosha, 2021). In this diabetic model, guar gum supplementation reduced serum creatinine, urea, and uric acid levels, likely through improved glycemic control, decreased oxidative stress, and modulation of inflammatory responses. Treatment with AEGN-3 at 25 and 50 mg/kg significantly improved renal biomarkers, reflecting substantial restoration of kidney function. This enhanced effectiveness is probably due to increased bioavailability, sustained drug release, and targeted delivery provided by the nanoformulation. Additionally, co-delivery allows

simultaneous modulation of oxidative stress, inflammation, and AGE-RAGE pathways, offering a comprehensive strategy to slow diabetic nephropathy progression. Overall, these findings highlight the therapeutic potential of AEGN-3 in managing diabetes-associated renal complications. The nanoformulation preserved renal function in HFD-fed, STZ-induced diabetic rats and may have broader applications for diabetic nephropathy via robust antioxidant and anti-inflammatory effects.

Hyperglycemia in STZ induced diabetes disrupts lipid homeostasis by upregulating key lipogenic enzymes such as acetyl CoA carboxylase and fatty acid synthase. Insulin resistance further impairs lipid uptake and metabolism in adipocytes and hepatocytes, causing elevated serum triglycerides, cholesterol, and lipoproteins (Uehara et al., 2023; Jiang et al., 2020; Shabalala et al., 2020). Under these conditions, the liver increases VLDL production, worsening triglyceride and cholesterol accumulation in circulation. This combined effect leads to elevated LDL and VLDL with a reduction in HDL, which is crucial for cholesterol efflux and vascular protection (Chao et al., 2021; Almalki et al., 2024). In agreement with these findings, STZ and HFD induced diabetic rats in our study exhibited dyslipidemia characterized by significant increases in total cholesterol, triglycerides, VLDL, and LDL, alongside decreased HDL. Acarbose, an α -glucosidase inhibitor, is known to improve lipid metabolism indirectly by reducing postprandial glucose and enhancing insulin sensitivity, which decreases hepatic lipid synthesis. Previous studies have shown that acarbose lowers cholesterol, triglycerides, VLDL, and LDL while raising HDL levels (Amashita et al., 1984; Carswell et al., 1989; Hung et al., 2022; Zhou et al., 2021). Consistent with these reports, our results demonstrated significant improvement in the lipid profile of diabetic rats treated with acarbose. Similarly, guar gum, a soluble dietary fiber, exerts antihyperlipidemic effects by delaying absorption of dietary lipids and cholesterol and promoting bile acid excretion. It has been reported to reduce total cholesterol, triglycerides, and LDL while increasing HDL in diabetic models (Samarghandian et al., 2012; Frias and Sgarbieri, 1998). In agreement, guar gum treatment in our study significantly improved the lipid profile. Importantly, AEGN-3 showed greater improvements in lipid parameters than acarbose or guar gum alone. This enhanced efficacy likely results from its ability to simultaneously target multiple metabolic disturbances characteristic of diabetic dyslipidemia. One mechanism may involve activation of AMP activated protein kinase, a central regulator of energy metabolism that promotes fatty acid beta oxidation, suppresses hepatic lipogenesis, and improves insulin sensitivity. These actions reduce very LDL production and enhance lipid clearance. Furthermore, the combination of

antioxidant and anti-inflammatory effects of guar gum with the antihyperglycemic effect of acarbose in AEGN-3 enables comprehensive regulation of lipid synthesis, oxidative stress, inflammation, and clearance. Thus, in agreement with the multifactorial nature of diabetic dyslipidemia, AEGN-3 offers a synergistic and integrated approach to restoring lipid balance and preventing cardiovascular complications in diabetes.

As previously described, the combination of STZ and HFD induces sustained hyperglycemia, insulin resistance, oxidative stress, and inflammation, leading to extensive organ damage. In the present study, significant histopathological alterations were observed in the liver, kidneys, pancreas, and heart of diabetic rats. The liver exhibited hepatocellular degeneration, cytoplasmic vacuolization, and inflammatory cell infiltration, indicative of the detrimental effects of chronic hyperglycemia and insulin resistance on hepatic tissue, consistent with previous findings in diabetic models (Açikel Elmas et al., 2020; Pilling et al., 2021; Barrière et al., 2018). Renal tissues exhibited glomerulosclerosis, tubular epithelial cell damage, and inflammatory infiltration, characteristic features of diabetic nephropathy, consistent with prior studies (Wu et al., 2016; Barrière et al., 2018; Kumari et al., 2021; Zhang et al., 2017; Cui et al., 2024). Pancreatic sections revealed beta cell destruction and islet atrophy, indicating impaired insulin secretion and metabolic dysfunction, which aligns with established pancreatic pathology in diabetes (El-Huneidi et al., 2022; Chen et al., 2022; Akinlade et al., 2021). The heart tissue demonstrated fragmentation of myocardial fibers and cytoplasmic vacuolization, changes indicative of diabetic cardiomyopathy and metabolic oxidative injury (Khanra et al., 2015; Barrière et al., 2018). Treatment with acarbose conferred moderate histological protection in the examined organs, primarily through the reduction of postprandial glucose levels, which attenuates glucotoxicity and subsequent oxidative stress and inflammatory responses. This is supported by earlier work showing acarbose reduces inflammatory markers, limits lipid accumulation in hepatic and renal tissues, preserves β -cell mass, and improves cardiac histopathology in diabetic conditions (Zhou et al., 2021; Wang et al., 2021).

Treatment with guar gum resulted in significant improvement in histopathological features, likely mediated by its capacity to enhance insulin sensitivity and slow nutrient absorption; these protective effects have been previously reported as reductions in hepatic steatosis, mitigation of renal oxidative stress, preservation of pancreatic islet integrity, and attenuation of myocardial fibrosis in diabetic models (Samarghandian et al., 2012; Frias and Sgarbieri, 1998). Importantly, AEGN-3 demonstrated the most pronounced histological improvements. This nanoformulation effectively restored hepatic architecture, reduced inflammatory cell

infiltration, preserved glomerular morphology, diminished tubular injury, promoted regeneration of pancreatic β -cells, and minimized myocardial vacuolization. The superior efficacy of AEGN-3 likely results from the combined pharmacological actions of acarbose and guar gum integrated into a nanoparticle delivery system. This platform facilitates enhanced bioavailability and targeted delivery, enabling concurrent modulation of multiple pathological mechanisms including oxidative stress, inflammation, insulin resistance, and protein glycation. Such multi-targeted therapeutic effects are supported by previous nanomedicine studies reporting improved multi-organ protection in diabetic models (Xie et al., 2018; Tsai et al., 2012). Collectively, these findings highlight AEGN-3 as a promising multifunctional therapeutic strategy for comprehensive management of diabetes-associated complications in critical organs including liver, kidneys, pancreas, and heart.

Chronic hyperglycemia impairs endogenous antioxidant defenses, thereby intensifying oxidative stress. Consistently, the HFD-fed, STZ-induced model in our study induced marked ROS production and a significant decline in the activity of key antioxidant enzymes such as SOD, catalase, and glutathione peroxidase, accompanied by elevated LPO. This redox imbalance aligns with earlier findings highlighting the synergistic effect of HFD and STZ in promoting systemic oxidative stress and suppressing antioxidant defenses (Ghosh et al., 2015; Asokan et al., 2019; Gilani et al., 2022; Lasker et al., 2019; Ghazal et al., 2024). In agreement with previous reports, our data confirm a profound oxidative environment in the liver, kidney, pancreas, and heart tissues of diabetic rats. Treatment with acarbose and guar gum notably ameliorated oxidative stress. Acarbose improved antioxidant enzyme activity and reduced LPO, aligning with prior studies demonstrating its efficacy in restoring redox balance and reducing inflammation (Garg et al., 2025). Clinical evidence further shows that acarbose co-administered with metformin outperforms glibenclamide in reducing oxidative stress in type 2 diabetic patients (Wang et al., 2011). Similarly, guar gum has shown significant antioxidative effects in HFD and galactose induced oxidative models by reducing LPO and improving antioxidant enzyme activities (Kuo et al., 2009; Liu et al., 2019). Its supplementation in aquatic models also led to improved antioxidant profiles (Zhao et al., 2024), and protection against vascular damage in thrombosis models (Kuo et al., 2009). In agreement with these results, our study demonstrates that both acarbose and guar gum independently improved oxidative stress markers across tissues. Notably, AEGN-3 exhibited the most pronounced protective effects, likely due to enhanced ROS scavenging facilitated by hydroxyl groups and the advantages of nanoparticle-based delivery, including targeted release and improved bioavailability. This is

consistent with recent advancements employing multifunctional nanocarriers in diabetes to address oxidative injury and metabolic complications. Overall, these findings support the therapeutic promise of AEGN-3 in restoring redox homeostasis and preserving tissue integrity in diabetes.

The present study demonstrated a significant elevation in xanthine oxidase and AChE activities in HFD-fed, STZ-induced diabetic rats, reflecting oxidative and cholinergic disturbances (Hernandez-Hernandez et al., 2022; Bravard et al., 2011). Xanthine oxidase catalyzes the oxidation of hypoxanthine and xanthine to uric acid, generating ROS as a by-product. Its activity is strongly upregulated under hyperglycemic and inflammatory conditions, contributing to tissue injury and progression of diabetic complications (Pacher et al., 2006; Desco et al., 2002). Likewise, AChE, which regulates acetylcholine turnover, becomes hyperactive under oxidative stress, disrupting cholinergic signaling and promoting neurodegenerative changes observed in diabetes (Marucci et al., 2021; Maciel et al., 2016). The observed increase in Xanthine oxidase and AChE activities supports the involvement of oxidative and neuronal dysfunction in diabetes. Earlier studies have shown that acarbose and guar gum can inhibit these enzymes and alleviate oxidative stress. Consistent with these reports, *Drosophila melanogaster* models treated with acarbose and natural polymers such as guar gum showed reduced Xanthine oxidase and AChE activities along with improved oxidative and inflammatory markers (Garg et al., 2025). Our *in vivo* findings confirmed that co-administration of acarbose and guar gum significantly lowered Xanthine oxidase and AChE levels in liver, kidney, pancreas, and heart, indicating restoration of redox balance. Among the formulations, AEGN-3 showed the most pronounced inhibitory effect on both enzymes. This enhanced activity may result from synergistic interaction between acarbose and guar gum and improved bioavailability achieved through nanoencapsulation. The nanoformulation likely enabled sustained release and better cellular uptake, thereby enhancing therapeutic efficiency. Overall, AEGN-3 appears to be a promising candidate for targeting oxidative and neurochemical imbalances associated with diabetes.

Inflammation is a key feature of diabetes, particularly in HFD-fed, STZ-induced models, where chronic hyperglycemia and lipid imbalance trigger elevated levels of pro-inflammatory cytokines such as TNF- α and IL-6, alongside reduced IL-10 (Tsalamandris et al., 2019; Rohm et al., 2022; Popa et al., 2007; Li et al., 2022). This inflammatory milieu contributes to insulin resistance, vascular dysfunction, and tissue damage (Lu et al., 2023; Yuan et al., 2019). Consistent with earlier studies, we observed significant increases in TNF- α and IL-6 with a

concomitant decrease in IL-10 in liver, kidney, pancreas, and heart tissues of diabetic rats, reaffirming the inflammatory nature of STZ and HFD induced diabetes (Mahmoud et al., 2012; Almalki et al., 2024; Lestari et al., 2023; Ghanbari and Sadeghimahalli, 2022; Roy et al., 2023; Al Trad et al., 2019). In agreement with these results, treatment with acarbose and guar gum led to a reduction in TNF- α and IL-6 levels and partial restoration of IL-10. These effects align with previous reports demonstrating their anti-inflammatory roles in diabetic settings (Zhao et al., 2020; Mo et al., 2019; Li et al., 2021; Paudel et al., 2024). Acarbose and guar gum likely exert these effects by modulating cytokine responses and mitigating oxidative damage. Notably, AEGN 3 showed a more substantial anti-inflammatory response than either acarbose or guar gum alone, as reflected by greater suppression of TNF- α and IL-6 and more effective restoration of IL-10. This enhanced activity may result from synergistic interactions within the nanoformulation, potentially improving bioavailability and cellular targeting. These results underscore the promise of AEGN-3 in attenuating diabetes related inflammation beyond glycemic control (Garg et al., 2025; Jhundoo et al., 2021).

Although acarbose primarily acts within the intestinal lumen as an α -glucosidase inhibitor and less than 2% of the drug enters systemic circulation (DiNicolantonio et al., 2015), accumulating evidence indicates that its therapeutic effects extend beyond local carbohydrate digestion. The reduction of postprandial hyperglycemia, modulation of gut-derived incretins, and alterations in gut microbiota and bile acid signaling can indirectly influence systemic pathways and gene expression. Moreover, several *in vivo* studies have reported changes in mRNA and protein levels (via RT-PCR and Western blotting) following acarbose treatment, which are interpreted as downstream regulatory consequences of these indirect mechanisms rather than direct intracellular drug binding. In this context, the network pharmacology approach provides valuable insight into the secondary molecular targets and signaling pathways potentially modulated by acarbose-mediated systemic effects.

In line with this perspective, we applied an integrative approach combining network pharmacology, target mining, enrichment analysis, PPI analysis, molecular docking, molecular dynamics, and RNA sequencing analysis using EasyGeo to further explore the mechanistic basis of acarbose in type 2 diabetes. Through this analysis, we identified ten key genes including AKT1, ALB, EGFR, ESR1, GSK3B, HSP90AA1, PPARG, SRC, STAT3, and TNF, which exhibited high hub and centrality values, suggesting critical roles in the diabetic network. These targets are involved in pathways related to insulin signaling, inflammation, and

metabolic regulation, indicating that acarbose may act through multiple mechanisms to exert broader therapeutic effects.

In the present study, several genes previously recognized as key contributors to type 2 diabetes were identified. SRC showed the highest association at 92.03% and is known to regulate glucokinase and insulin secretion, with its dysregulation linked to disease progression (Sato et al., 2016). AKT1, essential for glucose uptake and metabolism, is well established in insulin resistance, with a 90.77% association. GSK3B negatively regulates insulin signaling via IRS 1 and affects glucose homeostasis and lipid metabolism, making it a validated therapeutic target with an 83.13% association. ALB is involved in diabetic nephropathy through hyperglycemia-induced glomerular damage leading to albuminuria, showing an 82.33% involvement (Lim, 2014; Thipsawat, 2021). HSP90AA1 maintains protein stability and beta cell function, with its downregulation contributing to insulin resistance and inflammation, supported by a 79.59% association (Bellini et al., 2017; Jing et al., 2018). Other genes identified also display moderate to high relevance in type 2 diabetes, underscoring the complex molecular mechanisms of the disease and highlighting potential therapeutic targets.

In the present study, biological processes, molecular functions, and pathways associated with the targeted genes were analyzed through gene enrichment and PPI analyses (Yang et al., 2020; Yazdani et al., 2024). The enrichment analysis emphasized the key role of insulin receptor signaling, glucose homeostasis, and regulation of glucose transport in maintaining glucose balance and insulin sensitivity. It is well established that insulin receptor signaling activates insulin receptor substrate and the PI3K/Akt pathway, leading to glucose uptake (Huang et al., 2018; Savova et al., 2023; Schultze et al., 2012; Zhao et al., 2024). Disruptions in these pathways cause insulin resistance, impairing PI3K/Akt signaling, reducing glucose uptake, and lowering glycogen synthesis. This contributes to hyperglycemia and beta cell dysfunction. Additionally, insulin resistance promotes excessive hepatic glucose production, worsening diabetes progression (Zhao et al., 2023; Santoleri and Titchenell, 2019; Sebkova et al., 1995; Ormazabal et al., 2018). These findings align with previous reports highlighting the role of PI3K/Akt signaling in enhancing glucose uptake, suppressing gluconeogenesis in liver and muscle, and regulating lipid and glucose metabolism (Huang et al., 2018). Chronic low-grade inflammation in liver, muscle, and adipose tissue is also linked to insulin resistance, with TNF as a principal pro inflammatory cytokine activating JNK signaling, inhibiting GLUT4 translocation, and promoting insulin resistance (Nguyen et al., 2005; Chen et al., 2015; Castelli et al., 2024). Our results reinforce TNF induced inflammation as a key contributor to type 2

diabetes pathogenesis, suggesting TNF as a potential therapeutic target for diabetes related metabolic inflammation. Cellular component enrichment identified the insulin receptor complex as the most enriched site, critical for insulin signaling and glucose metabolism at the plasma membrane where insulin binding triggers downstream pathways such as PI3K/Akt. The cytoplasm contains proteins like AKT and PI3K essential for metabolic regulation (Hancock et al., 2019; Yunn et al., 2023), while the nucleus regulates genes involved in insulin signaling and metabolism, with dysfunction leading to impaired glucose homeostasis and inflammation. Molecular function enrichment underscored roles in insulin signaling including JUN kinase activity, insulin receptor activity, nitric oxide synthase regulator activity, insulin binding, and PI3K binding (Siddle, 2012). Functions such as insulin receptor binding, protein kinase binding, alpha amylase activity, and glucose transporter activity further support involvement in metabolic pathways relevant to diabetes mechanisms (Boucher et al., 2014; Frojdo et al., 2009; Chang et al., 2004; Lee et al., 2022; Batista et al., 2021).

A large body of research has demonstrated the critical role of PPI networks in connecting targeted proteins to biochemical processes involved in disease pathogenesis. Building on this, from an initial set of 127 genes, the top 10 genes were identified using degree, closeness, and betweenness metrics in Cytoscape. These genes were further confirmed for their relevance in liver, pancreas, adipose tissue, skeletal muscle, and blood of type 2 diabetes patients using clinical data from the GEO database. The findings support the involvement of these genes in the pathogenesis of type 2 diabetes and its complications. Molecular docking and dynamic simulation studies revealed strong binding affinity of acarbose to these key targets, with interactions at extensive active sites and stable binding throughout the simulations. However, to confirm the physiological relevance and therapeutic potential of these findings, further validation through gene expression analysis and investigation of downstream signaling pathways is necessary.

Chapter 8

Conclusion

This research aimed to overcome the limitations of conventional antidiabetic therapy by developing a novel guar gum-based nanoformulation of acarbose to improve its pharmacological efficacy, antioxidant potential, and biocompatibility through an advanced drug delivery system. Acarbose, a clinically approved α -glucosidase inhibitor, has limited effectiveness due to poor gastrointestinal absorption, low systemic bioavailability, and adverse gastrointestinal side effects such as abdominal discomfort, flatulence, and diarrhoea. Therefore, developing a delivery system that enables sustained release and targeted therapeutic effects was necessary to enhance glycemic control while minimizing side effects.

Comprehensive physicochemical characterization confirmed the successful preparation of nanoformulations with desirable particle size, surface charge, uniform morphology, and amorphous drug dispersion. These characteristics are essential for improving drug solubility and enhancing mucosal permeability. The nanoformulations demonstrated favourable drug entrapment efficiency and drug content, indicating that guar gum is an effective natural carrier for hydrophilic antidiabetic drugs. Molecular interactions between acarbose and guar gum helped stabilize the drug in a non-crystalline state, supporting improved dissolution and sustained release. *In vitro* release studies showed a sustained and controlled release pattern of acarbose from the nanoformulations compared to free drug. This sustained release is attributed to the hydrophilic and gel forming nature of guar gum, which modulates drug diffusion and retention in the gastrointestinal tract. This release behaviour may help reduce dosing frequency and improve drug tolerability by addressing the short half-life and poor absorption associated with conventional acarbose therapy.

Biological evaluations revealed significantly enhanced antioxidant and antidiabetic activities of the nanoformulations. They exhibited increased free radical scavenging ability across multiple assays and stronger inhibition of carbohydrate-digesting enzymes compared to native acarbose. These improvements are likely due to the enhanced bioavailability of acarbose in the nanoform and the inherent antioxidant properties of guar gum. The combined effects suggest a dual mechanism that slows carbohydrate digestion and reduces oxidative stress, providing a more comprehensive approach to managing type 2 diabetes.

Cell viability assays, along with LDH release and hemolysis studies, showed low cytotoxicity of the nanoformulations and confirmed their biocompatibility. These results demonstrated

protective effects on cellular integrity and minimal membrane damage, supporting their safety for oral administration. In models simulating diabetic conditions, the formulations reduced oxidative damage in a dose dependent manner, indicating enhanced cellular antioxidant defense likely due to the combined effect of acarbose and guar gum.

The therapeutic potential of the nanoformulations was further confirmed in a diabetic rat model induced by STZ and high fat diet. Treatment improved fasting blood glucose levels, restored antioxidant enzyme activities, and normalized renal function, lipid profiles, and organ histopathology. The nanoformulation also reduced common acarbose-related gastrointestinal side effects such as diarrhoea and decreased serum markers of liver and kidney damage. Oxidative stress and inflammatory markers in hepatic, renal, pancreatic, and cardiac tissues were significantly improved, demonstrating protection against diabetes related tissue damage.

Network pharmacology analysis revealed the involvement of diabetes-associated signaling pathways, including PI3K/Akt, AMPK, insulin signaling, and TNF pathways. Several key hub genes related to insulin sensitivity, beta cell function, inflammatory regulation, and cellular stress response were identified. Molecular docking and simulation studies demonstrated strong binding affinities between acarbose and these target proteins, indicating a multi-target modulatory mechanism contributing to the observed therapeutic effects.

In summary, this study shows that a guar gum-based nanoformulation of acarbose significantly enhances its therapeutic profile through sustained drug release, improved antioxidant capacity, and effective enzyme inhibition. The integration of experimental and computational data provides insight into the mechanisms of action. However, a limitation of the present study is that direct comparison with commercial sustained-release acarbose formulations was not performed; pure acarbose was used as a reference to illustrate immediate-release behaviour, effectively highlighting the sustained-release advantage conferred by the developed nanoformulations. Further pharmacokinetic evaluation, investigation of underlying molecular mechanisms to support mechanistic claims, experimental validation of molecular signaling pathways, and studies investigating the effects of the nanoformulation on gut microbiota are required to fully establish the clinical potential and systemic benefits. Additionally, future studies should evaluate formulation scalability, long-term stability, and perform comparative analyses with other polymer-based nanoformulations to strengthen translational relevance, along with exploring combination nanoformulations with multiple antidiabetic agents to further

enhance efficacy and safety. This work provides a strong foundation for developing safer and more effective oral therapies for type 2 diabetes and related metabolic disorders.

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Preparation, characterization, and pre-clinical evaluation of acarbose-guar gum solid dispersions in diabetic rats

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ABSTRACT

Acarbose, a clinically approved α -glucosidase inhibitor for type 2 diabetes, has limited efficacy due to gastrointestinal side effects, highlighting the need for delivery systems that modulate release and improve tolerability. This study aimed to develop and characterize acarbose-guar gum solid dispersions (AGSDs) and evaluate their physicochemical, *in vitro*, and *in vivo* properties. Solid-state characterization confirmed amorphous molecular dispersion with spherical particles and drug-polymer interactions. Among the developed solid dispersions, AGSD-3 showed the highest drug entrapment efficiency ($84.24 \pm 0.48\%$) and controlled release ($86.19 \pm 1.66\%$ over 24 h), compared to free acarbose. It exhibited strong enzyme inhibition with IC_{50} values of 6.97 ± 0.21 μ g/mL (α -glucosidase) and 12.86 ± 0.18 μ g/mL (α -amylase), along with favourable cytocompatibility and hemocompatibility. In high-fat diet (HFD)-fed, streptozotocin (STZ)-induced diabetic rat model, AGSD-3 at 50 mg/kg significantly reduced body weight and blood glucose ($p < 0.001$), while gastrointestinal side effects, such as diarrhoea, were limited to a single day (score 1), indicating improved tolerability compared to acarbose alone. Treatment also restored pancreatic weight ($p < 0.001$) and histology, and attenuated oxidative stress, and inflammation ($p < 0.001$), indicating protective effects against diabetes-induced tissue damage. Overall, these findings suggest that guar gum-based solid dispersion represents a promising strategy to enhance the therapeutic efficacy and tolerability of acarbose for diabetes management.

1. Introduction

The human body requires adequate amounts of nutrients to regulate metabolism, but when the body fails to process nutrients, it results in several metabolic disorders. Of these, type 2 diabetes is the most prevalent, resulting from defects in either insulin resistance, insulin secretion, or both [1]. It is well known that type 2 diabetes causes many systemic complications such as neuropathy, cardiac disorders, and increased oxidative stress, which consequently affect the quality of life of patients and impose huge healthcare expenditures globally [2].

Postprandial glycemic control through inhibition of the α -glucosidase enzyme in the intestine is one of the key strategies to manage diabetes [3]. Acarbose is a complex oligosaccharide, and an antidiabetic agent widely used to control postprandial hyperglycemia. It acts primarily as a competitive inhibitor of the intestinal brush border enzyme α -glucosidase. This results in a slowing of the enzymatic breakdown of

complex carbohydrates in the diet into monosaccharides, delaying the intestinal absorption of glucose. Thus, acarbose helps to reduce the postprandial rise in blood sugar levels after meals and provides an effective therapeutic option in the management of type 2 diabetes [3,4].

Although acarbose has been shown to be an effective anti-diabetic agent, its clinical use is limited due to some limitations and side effects associated with conventional therapy. These mainly include inadequate absorption in the digestive tract, short half-life, and low oral bioavailability. Acarbose usually needs to be given in frequent doses (three times a day, approximately 25–100 mg per dose) to adequately control glucose levels. Gastrointestinal side effects such as flatulence and diarrhoea are frequently observed with this high dosing frequency, which affects its tolerability and safety profile [5,6]. Thus, it is imperative to look for an alternative drug delivery system that can reduce the side effects associated with conventional acarbose therapy while also enhancing its pharmacological effects.

In recent years, drug development research has increasingly focused

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Enhancing Anti-Diabetic effects of acarbose with natural Polymers: Mitigation of oxidative stress and inflammatory markers in *Drosophila melanogaster* (Oregon R + Strain)

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ABSTRACT

Acarbose, an α -glucosidase inhibitor with poor bioavailability, benefits from combination with natural polymers to improve stability, bioavailability, and antioxidant effects. This study evaluated acarbose combined with pectin, β -cyclodextrin, and guar gum at various ratios for antidiabetic and antioxidant potential. In silico studies indicated strong binding energy and stability, while in vitro tests confirmed enhanced enzyme inhibition, radical scavenging, and metal chelation. Using 1% hydrogen peroxide (H_2O_2) in *Drosophila* larvae, the 50:50 combination with pectin and guar gum demonstrated superior antioxidant effects, reduced lipid peroxidation, increased antioxidant enzyme activity, and modulated inflammation by reducing $TNF-\alpha$ and enhancing IL-10 levels. This combination shows promise for further optimization in nanodelivery systems.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to impaired insulin secretion and resistance. Its pathophysiology involves dysregulation of PI3K/Akt, AMPK, MAPK, and NF- κ B pathways, leading to oxidative stress, inflammation, and β -cell dysfunction, which cause complications like neuropathy, nephropathy, and impaired wound healing [1,2]. Diabetes also contributes to cognitive decline and neurodegeneration through chronic inflammation and oxidative damage [3]. Impaired wound healing results from dysregulated inflammation, reduced angiogenesis, and biofilm formation [1]. Various therapeutic approaches, including antioxidant and anti-inflammatory agents, neuroprotective compounds, and drug delivery systems, have been explored to mitigate these complications and enhance treatment efficacy.

Acarbose inhibits α -glucosidase, delaying carbohydrate digestion and reducing postprandial glucose spikes. However, its limitations, such as a short half-life and low oral bioavailability, affect its effectiveness. Pharmacological management of diabetes aims to maintain blood glucose levels, but conventional treatments often struggle with these

challenges, necessitating advanced drug delivery strategies. To achieve therapeutic efficacy, acarbose requires frequent administration, often leading to gastrointestinal side effects [4]. Recent advancements in plant-based polysaccharides and nanotechnology-based strategies have been widely used to enhance bioavailability, regulate blood glucose levels, reduce oxidative stress and gastrointestinal issues [5]. Additionally, the combination of bioactive compounds like gallic acid with acarbose has shown promising results in enhancing both antidiabetic and antioxidant properties [4]. This study explores acarbose-polymer combinations to enhance therapeutic efficacy, stability, and antidiabetic effects in a *Drosophila* oxidative stress model.

2. Material and methods

In this study, all chemicals were of analytical grade from Hi Media, except Sodium fluoride (CAS:7681-49-4), Phenylmethylsulfonyl fluoride (CAS:329-98-6), NBT (CAS:298-83-9), Hydroxylamine hydrochloride (CAS:5470-11-1), Acetylthiocholine (CAS:1866-15-5), DTNB (CAS:69-78-3), which were from Sigma.

Molecular docking studies were conducted using AutoDock Vina to

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α -Glucosidase inhibitor: identifying key targets and mechanisms in type 2 diabetes

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Abstract

Acarbose is an α -glucosidase inhibitor that helps lower blood sugar after meals by stopping complex carbohydrates from turning into simple sugars; however, its role other than α -glucosidase inhibition is not yet fully understood. In this study, we used bioinformatics to explore the molecular targets and mechanisms of acarbose in type 2 diabetes (T2D). We found one hundred and twenty-seven shared targets between proteins linked to acarbose and genes related to T2D. Analysis showed these targets are mainly involved in insulin signaling, glucose metabolism, PI3K/Akt pathway, and inflammation. Network analysis highlighted 10 important genes: AKT1, ALB, EGFR, ESR1, GSK3B, HSP90AA1, PPARG, STAT3, SRC, and TNF. Expression profiling showed these genes have different patterns in various tissues from diabetic samples. Molecular docking results indicated a binding affinity of acarbose with the key proteins, with EGFR showing the lowest binding energy of -7.8 kcal/mol. Molecular dynamics (MD) simulations confirmed that the acarbose-protein complexes, especially with EGFR, STAT3, PPARG, and HSP90AA1, are stable. Overall, these results provide a detailed information on how acarbose may act on multiple targets other than inhibiting α -glucosidase in T2D.

Keywords Acarbose · Type 2 diabetes · Network pharmacology · Molecular docking · Insulin signaling pathway

Introduction

Type 2 diabetes (T2D) is a long-term metabolic disorder marked by insulin resistance and reduced insulin secretion,

which causes ongoing high blood sugar. As pancreatic β -cell function slowly declines, blood sugar control worsens. The International Diabetes Federation reports that over 529 million adults worldwide have diabetes, and more than half of these cases are undiagnosed. Many cases go undetected because the disease often develops quietly at first, there is limited access to regular screening, and public awareness is low (Accili et al. 2025). Countries like China, India, and Pakistan make up a large share of this global problem, mainly because of their big and aging populations, fast urbanization, less physical activity, and increasing rates of obesity and dietary changes (Garg et al. 2024; Shukla et al. 2026; Malik, et al. 2025).

Many preclinical and clinical trials have investigated several candidate natural and synthetic bioactive compounds for their effectiveness in controlling blood glucose levels. These efforts have helped identify several promising candidates that have moved into clinical trials focused on managing hyperglycemia (Rahman et al. 2022). Acarbose is one such compound that has proven effective in lowering blood glucose levels. It inhibits brush-border α -glucosidase

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OPEN Sinapic acid accelerates diabetic wound healing by promoting angiogenesis and reducing oxidative stress

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The incidence of delayed wound healing associated with diabetes is increasing globally. Synthetic drugs for wound management often carry adverse effects, underscoring the need for safer and more effective alternatives. Sinapic acid, a phytochemical found in edible plants such as spices, citrus fruits, and berries, has drawn attention for its potential in addressing diabetic wounds. This study investigates the protective effects of sinapic acid at two pharmacological doses in mitigating delayed wound healing and oxidative stress linked to type 2 diabetes. In-vitro, the effects of sinapic acid on cell toxicity, migration, and antioxidant activity were evaluated under high-glucose conditions using L929 murine fibroblast cells and human umbilical vein endothelial cells (HUVEC). In-vivo, diabetic wounds were induced in male Sprague Dawley rats fed with high-fat diet and treated with streptozotocin. Sinapic acid was administered orally at 20 mg/kg and 40 mg/kg, and its efficacy was assessed using an excision wound model. Key markers, including hepatic, and lipid profiles, were analyzed. The findings revealed that sinapic acid significantly improved blood glucose levels and oxidative stress markers in diabetic wound rats. Enhanced angiogenesis, re-epithelialization, wound contraction, cell migration, and SIRT1 levels were observed. This is the first report demonstrating that oral sinapic acid at 20 mg/kg and 40 mg/kg mitigates oxidative stress and promotes wound healing in streptozotocin/high-fat diet (STZ/HFD)-induced diabetes, with lower doses showing greater efficacy.

Keywords Diabetes, Delayed wound healing, Sinapic acid, Angiogenesis, Oxidative stress

Abbreviations

ALP	Alkaline phosphatase
BME	Basement membrane extract
BSA	Bovine serum albumin
CAT	Catalase
CMC	Carboxymethylcellulose
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulphoxide
DPPH	1,1-Diphenyl-2-picrylhydrazyl
GSH	Reduced glutathione
GST	Glutathione S Transferase
H&E	Hematoxylin and eosin
HDL	High-density lipoprotein
HFD	High-fat diet
HUVEC	Human umbilical vein endothelial cells
LDL	Low-density lipoprotein

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RESEARCH ARTICLE



Screening of natural epigenetic modifiers for managing glycemic memory and diabetic nephropathy

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ABSTRACT

Short hyperglycaemic episodes trigger metabolic memory (MM) in which managing hyperglycaemia alone is not enough to tackle the progression of Diabetic nephropathy on the epigenetic axis. We used a structural similarity search approach to identify phytochemicals similar to natural epigenetic modifiers and docked with SIRT1 protein and did ADME studies. We found that UMB was 84.3% similar to esculetin. Upon docking, we found that UMB had a binding energy of -9.2 kcal/mol while the standard ligand had -11.8 kcal/mol. ADME showed UMB to be a good lead. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay showed it to be a good antioxidant with IC_{50} of 107 μ g/mL and MTT stands for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) showed that it does not promote cell death. Oxidative biomarkers *in vitro* showed UMB was able to ameliorate glycemic memory induced by high glucose. Western blot revealed decreased histone acetylation under hyperglycaemic conditions and upon treatment with UMB along with DR, its levels increased. This led us to check our hypothesis of whether concomitant diet reversal (DR) together with UMB can alleviate high-fat diet-induced metabolic memory and diabetic nephropathy (DN) in SD rats. UMB was able to decrease blood glucose, lipid, renal, and liver profile concluding UMB was able to ameliorate DN and MM by increasing the histone acetylation level.

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Hyperglycaemia; metabolic memory; histone acetylation; oxidative biomarkers; diet reversal; diabetic nephropathy

Introduction

The Diabetes Control and Complications Trial (DCCT), and the follow-up study Epidemiology of Diabetes Interventions and Complications (EDIC) highlighted the benefit of strict glycemic control. Transient episodes of hyperglycaemia can cause long-lasting effects even after good glycemic control has been achieved, which is indicative of 'metabolic memory' (MM) [1]. Metabolic memory, also known as the legacy effect, refers to the phenomenon where prior exposure to high blood glucose levels can have a long-term impact on the risk of developing complications, even if blood glucose levels are subsequently brought under control. The mechanisms underlying metabolic memory are complex and multifactorial, but there are several proposed explanations [2]. One proposed mechanism is glucotoxicity, which refers to the toxic effects of high blood glucose levels on cells and tissues. Glucotoxicity can lead to irreversible damage to mitochondria and blood vessels, which can contribute to the development of long-term complications such as diabetic retinopathy, neuropathy, and nephropathy. In addition, glucotoxicity can cause long-lasting changes in the epigenetic regulation of key inflammatory mediators, which can contribute to the development of chronic inflammation, and diabetes and further exacerbate the risk of complications like diabetic nephropathy (DN) [3].

Another proposed mechanism involves short-term hyperglycaemia inducing long-term changes in chromatin modifications.

Chromatin modifications refer to changes in the structure of DNA and its associated proteins, which can affect gene expression [4]. Short-term exposure to high blood glucose levels can lead to changes in chromatin modifications that persist even after blood glucose levels are normalised [5]. SIRT1 inhibitors have been reported to lower kidney fibrosis activation. [6,7]. EX-527, a novel SIRT1 inhibitor, limits interstitial fibroblast activation in renal interstitial fibroblast cell line and reduces the expression of fibroblast activation markers [8]. Understanding these mechanisms is important for developing effective interventions to prevent or mitigate the long-term complications associated with diabetes. Metformin has been used extensively to treat diabetes and diabetes-related complications and has pleiotropic effects. A study done by Tikoo et al. 2016 [9] on metformin elucidated the role of metformin in ameliorating glycemic memory. But metformin being a synthetic drug is loaded with side effects like nausea, flatulence, diarrhoea, ingestion, abdominal discomfort, and vomiting [10,11]. Natural epigenetic modifiers have been a subject of research for the last decade. Phytochemicals that have been used in treating diabetes and its complications have already been discussed in detail [12].

Since only long-term blood glucose control can inhibit the progression of diabetic complications, the need of the hour is to look for alternatives that are not synthetic because we want to reduce the load of the side effects, so phytochemicals can be a good target. To achieve this goal, we did structural similarity searching of known epigenetic modifiers to find phytochemicals

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RESEARCH ARTICLE



Exogenous GABA as a natural epigenetic modifier for managing glycemic memory and diabetic nephropathy by modifying the epigenetic axis

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ABSTRACT

Background: Glycemic memory contributes to the progression of diabetic nephropathy (DN) despite glycemic control. This study investigates γ -aminobutyric acid (GABA), a natural compound with 82.5% structural similarity to metformin, for its potential in mitigating glycemic memory and DN.

Methods: Structural similarity and molecular docking identified GABA as a SIRT1-targeting metformin analog (binding affinity: 5.8 kcal/mol), supported by ADME profiling. *In vitro* assays assessed antioxidant activity (DPPH IC₅₀: 141.09 μ g/mL), cytotoxicity (MTT assay), oxidative stress markers, and histone H3 acetylation. *In vivo*, high-fat diet-fed Sprague-Dawley rats underwent dietary reversal and GABA treatment (100/200 mg/kg) to evaluate metabolic, renal, hepatic, oxidative, and epigenetic effects.

Results: GABA maintained >90% cell viability at 5 μ M, with no cytotoxicity up to 150 μ M. It reduced oxidative markers and restored histone acetylation *in vitro*. *In vivo*, 200 mg/kg GABA treatment significantly reduced cholesterol (44.44%), triglycerides (28.64%), and LDL (40.80%), while increasing HDL by 103.65%. At 100 mg/kg, GABA lowered blood urea (30.43%), creatinine (4.65%), uric acid (75.00%), bilirubin (53.57%), SGOT (54.24%), SGPT (39.52%), and ALP (60.58%), with histopathological improvements in renal tissues.

Conclusion: GABA exhibits antioxidant, hepatoprotective, and renoprotective properties, highlighting its potential as a therapeutic agent for glycemic memory-associated DN.

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Diabetes; glycemic memory; diabetic nephropathy; GABA; oxidative stress; histone acetylation; SIRT1; diet reversal

Introduction

Diabetes is a chronic metabolic disorder that significantly impacts global health, characterised by persistent hyperglycaemia resulting from insulin deficiency, insulin resistance, or a combination of both [1,2]. The two primary forms, type 1 diabetes and type 2 diabetes, differ in aetiology but ultimately lead to long-term complications affecting multiple organ systems. Type 1 diabetes results from autoimmune-mediated destruction of pancreatic β -cells, whereas type 2 diabetes is primarily associated with insulin resistance and β -cell dysfunction, often influenced by lifestyle and genetic predispositions. Type 1 diabetes is commonly linked to acute complications such as diabetic ketoacidosis, while type 2 diabetes is more frequently associated with metabolic syndrome, including obesity, hypertension, and dyslipidemia [1,3]. Both forms contribute to the development of chronic complications such as diabetic nephropathy, neuropathy, retinopathy, and cardiovascular disease, although the underlying mechanisms and progression may differ [4]. Despite significant advancements in diabetes diagnosis and treatment, the burden of these complications remains a major clinical challenge [4].

Emerging research has highlighted the concept of metabolic memory, also referred to as the legacy effect, which suggests that prior episodes of hyperglycaemia can have lasting effects even

after glycemic control is achieved [5]. This phenomenon underscores the importance of early and sustained intervention in diabetes management. One of the primary mechanisms driving metabolic memory is glucotoxicity, in which chronic hyperglycaemia induces oxidative stress, mitochondrial dysfunction, and vascular damage [6,7]. These pathological changes contribute to the progression of diabetic complications even in individuals who achieve subsequent glycemic control. Additionally, prolonged hyperglycaemia triggers persistent epigenetic modifications, including DNA methylation, histone modifications, and chromatin remodelling, which regulate gene expression and cellular function long after glucose levels normalise. These alterations affect key metabolic and inflammatory pathways, resulting in sustained cellular dysfunction and the progression of complications [7].

Among these epigenetic modifications, histone acetylation plays a crucial role in the development of diabetes-related complications. Hyperglycaemia-induced changes in histone acetylation persist due to the recruitment of histone deacetylases and dysregulation of histone acetyltransferases (HATs), collectively maintaining a pro-inflammatory and fibrotic state even after glucose levels return to normal [8–10]. Some reports have highlighted that elevated blood glucose levels can lead to an increase in histone deacetylase activity and a corresponding decrease in histone acetyltransferase activity in the retina and retinal

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Review

Guar gum-based nanoformulations: Implications for improving drug delivery

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ABSTRACT

Poorly soluble drugs are reported to easily degrade in the gastrointestinal tract and contribute in limiting the effect of drug to its targeted site. Oral administration of drug is one of the prominent ways to deliver a drug, although, it experiences barriers like acidic pH, presence of microflora and enzymes in the gastrointestinal tract. Collectively all of these participate in the degradation of drug before it reaches its target site and thus, they impede the sustained effect of drug. A quest of choosing a polymer with good stability profile and releasing the drug to its targeted site is always been a challenge for the scientists worldwide. Many polymers have been reported to prevent the degradation of drug and one such naturally occurring biocompatible polymer is guar gum. Guar gum-based nanoformulations have been extensively used in past decades to achieve controlled drug release which defines its importance. The coating of guar gum over the drug improves the bioavailability of the drug and thus helps in minimizing the risk of drug degradation. This review intends to highlight the beneficial role of guar gum-based nanoformulations to improve drug delivery by ameliorating the bioavailability.

1. Introduction

Polymers impersonate an influential role in the successful delivery of a drug [1]. Polysaccharides are the universal polymers obtained from nature. The polysaccharides have been broadly used because they manifest high bioavailability, are easily available, biodegradable, and environmental friendly. More importantly, they are non-toxic so that they do not cause any damage to the tissue or organ and do not interfere with the functioning of any organ. Chemical compounds that form a long structure when coupled through glycosidic bonds are called polysaccharides. There are several ways available that can be used to classify the nature of polysaccharides. These methods include the source, molecular weight, and hydrophilicity [2]. Guar gum is a naturally occurring polymer that does not corrode easily [3], which makes it proficient for effective drug delivery in targeted area. The stomach has a highly acidic pH, due to which when any drug is consumed through the oral route; the drug undergoes degradation as it reaches the acidic pH of stomach [4]. In addition to the acidic effect, microorganisms present in the small intestine also spoil the drug [5]. Therefore, polymers such as guar gum are used to prolong the stability of the drugs in the gastrointestinal tract.

Guar gum is heavily used by pharmaceutical industries to make an

effective nanoformulation with potential to beat the obstacles faced by drug when taken orally. Any polymer used for drug delivery must be a good binder (highly adhesive), stabilizer, disintegrating agent, and emulsifier [6]. Guar gum meets all the conditions to act as an efficient polymer and to overcome the barriers of drug delivery. Studies have proven that consumption of guar gum helps in controlling diabetes [7]. Additionally, guar gum also possesses activities like anti-inflammatory, anti-proliferative and anti-microbial activities [8,9]. Among all other reagents, guar gum shows high solubility in water and its endosperm contains galactomannan gum, which combines with water to form a viscous solution. In the food industry, this viscous solution creates a lot of constraints when consumed as dietary fiber [10]. In pharmaceutical industries its perpetual use has been to modify the excipient profile with the help of grafting, network formation, and derivatization which allow its use in drug delivery [11].

Reports have revealed that when a mixture of guar gum and water is heated, the water molecule that surrounds the guar gum loses its order [12]. Due to which the conformational changes occur, and the viscosity of guar gum decreases in hot water. When blended with water, the viscosity rate of guar gum increases, reducing the employment and effectiveness of lipids and proteins. These aspects fundamentally

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Review

Polyol pathway and redox balance in diabetes

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ARTICLE INFO

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ABSTRACT

Diabetes is a major public health disease that is globally approaching epidemic proportions. One of the major causes of type 2 diabetes is either a defect in insulin secretion or insulin action which is usually caused by a combination of genetic and environmental factors. Not only these factors but others such as deregulation of various pathways, and oxidative stress are also known to trigger the redox imbalance in diabetics. Increasing evidences suggest that there are tight interactions between the development of diabetes and redox imbalance. An alternate pathway of glucose metabolism, the polyol pathway, becomes active in patients with diabetes that disturbs the balance between NADH and NAD⁺. The occurrence of such redox imbalance supports other pathways that lead to oxidative damage to DNA, lipids, and proteins and consequently to oxidative stress which further ascend diabetes and its complications. However, the precise mechanism through which oxidative stress regulates diabetes progression remains to be elucidated. The understanding of how antioxidants and oxidants are controlled and impact the generation of oxidative stress and progression of diabetes is essential. The main focus of this review is to provide an overview of redox imbalance caused by oxidative stress through the polyol pathway. Understanding the pathological role of oxidative stress in diabetes will help to design potential therapeutic strategies against diabetes.

1. Introduction

Glycolysis is one of the important metabolic processes that occur in the cytoplasm of a cell with the aim to convert glucose into two molecules of pyruvate. It is known to occur in both aerobic and anaerobic organisms and does not require oxygen for its function. An essential enzyme in glycolysis that allows cells to use glucose as an energy source through phosphorylation is called hexokinase. The presence of excessive glucose in the cells results in the saturation of hexokinase further allowing the entry of glucose through a pathway called the polyol pathway [1]. Once glucose is ingested, key enzymes present in the polyol pathway perform their function such as converting glucose into sorbitol with the help of aldose reductase (AR) utilizing a nucleotide adenine dinucleotide phosphate (NADPH) [2]. One another important enzyme that mediates the conversion of sorbitol into fructose is called sorbitol dehydrogenase (SD). This conversion also leads to the formation of nicotinamide adenine dinucleotide hydrogen (NADH) from

nicotinamide adenine dinucleotide (NAD) [3]. The main function of NADPH is to mediate the reduction of glutathione followed by the production of nitric oxide (NO). But changes in the concentration of NADPH result in either a decrease in the content of glutathione or a deficiency thereof. The glutathione deficiency triggers a cascade of oxidative stress (OXS) and thus supports hemolysis in affected individuals [4].

Of all other metabolic disorders, diabetes contributes most to the body by down or upregulating the various metabolic pathways. As AR is found predominantly in tissues like the retina, lens, nerve, vascular, and glomerulus, the polyol pathway also aids in the progression of retinal, nerve, and kidney-related microvascular complications in diabetic patients [5]. In other words, the polyol pathway is responsible for cell damage through the formation of reactive oxygen species (ROS). Not only ROS are primarily involved in this process, but there are also other factors such as elevated levels of sorbitol and decreased levels of glutathione and NO. AR shows a very low affinity for glucose and

Abbreviations: AR, Aldose reductase; SD, Sorbitol dehydrogenase; NADH, Nicotinamide Adenine Dinucleotide Hydrogen; NAD, Nicotinamide Adenine Dinucleotide; NADPH, Nucleotide Adenine Dinucleotide Phosphate; NO, Nitric oxide; OXS, Oxidative stress; ROS, Reactive oxygen species; GSSG, Oxidised glutathione; GSH, Reduced glutathione; ATP, Adenine triphosphate.

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Review article

Cubosomes: An emerging nanodrug delivery platform for anti-diabetic medications

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ABSTRACT

Cubosomes are nanostructured particles characterized by a crystalline lyotropic liquid core that can be formulated by amphiphilic lipids with the capability of natural self-assembly in water and stabilized by poloxamers. A thermodynamically stable colloidal dispersion is produced by the cubic phase. Cubosomes have emerged as promising drug delivery system for administering oral drugs as they possess many benefits like the ability to load hydrophobic as well as hydrophilic active pharmaceutical ingredients, high encapsulation efficiency for drugs, targeted and sustained drug release, lipids biodegradability and simple technique of preparation. Numerous studies have explored the use of cubosomes to enhance the bioavailability of drugs with low water solubility. Due to the shorter half-life and poor bioavailability of many anti-diabetic drugs, prior investigations have highlighted the effectiveness of cubosomal nanoparticle formulations in efficiently delivering antidiabetic drugs through oral and intraperitoneal routes. Therefore, in this review article, the pertinent literature on cubosomes self-assembling, composition, preparation methods, and application for the delivery of anti-diabetic drugs is critically reviewed. Additionally, we discuss that tremendous progress has been made in the use of delivering drugs using other nanodelivery systems; however, there is a great need for progress in the delivery of anti-diabetic drugs using cubosomes.

1. Introduction

Diabetes stands out as a significant global public health concern, affecting a substantial portion of the world's population. The primary characteristic of diabetes mellitus is elevated blood sugar levels, resulting from either inadequate insulin secretion or the inability of the pancreas to produce insulin [1]. According to the latest data from the International Diabetes Federation, the current number of people worldwide living with diabetes exceeds 541 million. Projections indicate a further increase to 643 million by 2030 and 783 million by 2045 [2]. Globally, India, China, and the United States stand at the forefront with the highest rates of diabetes prevalence and associated mortality [3]. The ongoing surge in diabetes cases inevitably elevates the global diabetes mortality index, which recorded 6.7 million deaths in 2021 [2]. This trend poses a substantial economic and health burden on the world, currently nearing one trillion USD, and projections indicate a further increase by 2030 [4]. Individuals with diabetes are advised to adopt a nutritious diet and engage in regular physical activity to manage elevated blood sugar levels. However, in cases where diet and lifestyle

adjustments prove ineffective in normalizing glucose levels, diabetic individuals are compelled to initiate anti-diabetic therapy.

The oral administration of drugs continues to be a prevalent method for delivering medications, owing to its patient-friendly nature, non-invasive approach, cost-effectiveness, and the convenience of large-scale production [5]. This delivery approach is utilized to address a variety of conditions, including both systemic and local gastrointestinal disorders. About 40 % of emerging drug candidates, capable of enhancing disease conditions, experience physiological and physico-chemical conditions within the gastrointestinal tract. This encompasses the highly acidic pH of the stomach and the presence of intestinal microbes or mucus. Collectively, these biological barriers adversely impact drug delivery by reducing half-life, bioavailability, solubility, and hastening the elimination period [6]. The substantial rise in global diabetes cases calls for an alternative strategy. However, the discovery and validation of a novel bioactive compound by researchers is a time-consuming process. Therefore, it is recommended to focus on improving the shortcomings of those chemical compounds that have already been validated for their potential and are currently in clinical

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Review article

Recent advances in polymer-based nanoformulations for enhancing oral drug delivery in diabetes

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ABSTRACT

Diabetes is a long-term metabolic disorder caused by issues with insulin production or response in the body. This leads to high levels of glucose in the blood, which can cause other health problems. People with diabetes are advised to eat healthily and stay active to manage their blood sugar levels. However, if these lifestyle changes aren't enough, they may need to take medication. The current oral medications for diabetes have limitations, including poor solubility and low effectiveness. To address these issues, scientists are developing nanoformulations using large molecules. These nanoformulations have shown promise in improving the effectiveness of diabetes drugs by making them easier to absorb, reducing the required dosage, and increasing their solubility in water. While, research has shown the potential benefits of nanoformulations, more studies are needed before they can be used in clinical trials and approved for widespread use. This review explores different nanoformulations for treating diabetes and discusses how they could enhance the clinical effectiveness of anti-diabetic medications. Nevertheless, these advancements offer hope for better managing diabetes and reducing its impact on global health.

1. Introduction

Obesity, diabetes, and cardiovascular disorders are common lifestyle disorders that occur as a result of changes in food eating, behaviour, and lack of regular exercise [1,2]. Amongst these disorders, diabetes is a major public health challenge that impacts the world population on a great scale [3–5]. The major hallmark of diabetes mellitus is hyperglycemia which is characterized by defects in insulin secretion or by the failure of the pancreas to produce insulin [5–12]. The most recent statistical report from International Diabetes Federation indicates that there are currently over 541 million individuals living with diabetes worldwide, and this figure is expected to rise to 643 million by 2030 and 783 million by 2045. Compared to women, diabetes mellitus mainly affects men with higher incidence, and it is more prevalent in low- and middle-income countries [13]. Worldwide India, China, and the United States are the leading countries with the highest prevalence of diabetes

and the highest incidence of diabetes-associated mortality [14–20]. The continuous increase in patients with diabetes will automatically increase the global diabetes-mortality index (6.7 million deaths in 2021), which will have an even greater economic and health burden on the world as it is currently close to one trillion USD and is projected to increase by 2030 [13,21].

Several reports have confirmed that under hyperglycemic conditions, there is an increased ability/transition of glucose to get converted into enol which is called glucose autooxidation [22–25]. This reaction serves as a substantial source of free radicals such as hydrogen peroxide and superoxide anion which ultimately cause oxidative damage to protein, cell membrane, and DNA [26–28]. Furthermore, free radical generation was found to decrease the activity of antioxidant defense enzymes such as catalase, superoxide dismutase, and reduced glutathione [29–35]. Since diabetes and diabetes-induced oxidative stress are closely linked, thus intruding this link is of utmost importance to reduce

Abbreviations: TNF- α , Tumor necrosis factor- α ; IL-6, Interleukin-6; EGCG, Epigallocatechin gallate; AMPK, AMP-activated protein kinase; GLP-1, Glucagon-like peptide-1; VEGF, Vascular endothelial growth factor.

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Review

Biological macromolecules-based nanoformulation in improving wound healing and bacterial biofilm-associated infection: A review

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ABSTRACT

A chronic wound is a serious complication associated with diabetes mellitus and is difficult to heal due to high glucose levels, oxidative stress, and biofilm-associated microbial infection. The structural complexity of microbial biofilm makes it impossible for antibiotics to penetrate the matrix, hence conventional antibiotic therapies became ineffective in clinical settings. This demonstrates an urgent need to find safer alternatives to reduce the prevalence of chronic wound infection associated with microbial biofilm. A novel approach to address these concerns is to inhibit biofilm formation using biological-macromolecule based nano-delivery system. Higher drug loading efficiency, sustained drug release, enhanced drug stability, and improved bioavailability are advantages of employing nano-drug delivery systems to prevent microbial colonization and biofilm formation in chronic wounds. This review covers the pathogenesis, microbial biofilm formation, and immune response to chronic wounds. Furthermore, we also focus on macromolecule-based nanoparticles as wound healing therapies to reduce the increased mortality associated with chronic wound infections.

1. Introduction

Diabetes is a metabolic disorder that is characterized by an increased plasma glucose level that either results from defects in insulin action, insulin secretion, or both. A current survey by International Diabetes Federation confirmed that throughout the world, around 537 million individuals are affected with diabetes and it is expected that the figure will rise up to 643 million by 2030 and 783 million by 2045 [1]. Patients with diabetes are at increased risk of chronic wound development, particularly in the foot area, also called diabetic foot ulcers (DFUs) [2]. Approximately 15–20 % of patients with diabetes develop DFU annually and almost 15 % of the health budget yearly is used to treat patients with diabetes and its complications (DFU patients) worldwide. The decreased glucose metabolism was thought to affect the wound-healing process in patients with diabetes thereby installing chronic wounds [3]. The elevated level of blood glucose leads to poor blood flow due to which essential nutritional components are unable to reach the wounded area and impair the healing mechanism [4]. Chronic inflammation and increased oxidative stress are characteristics of chronic wounds that result in causing extensive host tissue damage. For example,

polymorphonuclear neutrophils (PMNs), an immune cell serves as potent effectors of inflammation as they display first-line defense in case of tissue injury. Nevertheless, excessive PMN activation results in increased reactive oxygen species production at the site of inflammation, which affects the function of the vascular endothelium. The main function of the vascular endothelium is to mediate the movement of inflammatory cells from blood to tissue. However, in inflammatory conditions, PMN-stimulated oxidative stress opens the inter-endothelial junction and facilitates the migration of inflammatory cells across the endothelial layer and as a result, they lead to tissue injury [5,6]. Therefore, disrupting the link between chronic inflammation and PMNs is imperative to reduce the increased incidence of diabetes-associated chronic wounds.

Human skin is an important interface between the environment and the human body and is inhabited by diverse and complex microbial communities, whose health impacts are still not clear [7,8]. Opportunistic pathogens although a part of the normal flora of the skin, are also responsible for causing infection during diabetic conditions whereas commensal bacteria in general stimulate innate and adaptive immune responses and prevent pathogenic infections [9,10]. The presence of

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Association between obesity, inflammation and insulin resistance: Insights into signaling pathways and therapeutic interventions

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ABSTRACT

Obesity, a metabolic disorder, is becoming a worldwide epidemic that predominantly increases the risk for various diseases including metabolic inflammation, insulin resistance, and cardiovascular diseases. However, the mechanisms that link obesity with other metabolic diseases are not completely understood. In obesity, various inflammatory pathways that cause inflammation in adipose tissue of an obese individual become activated and exacerbate the disease. Obesity-induced low-grade metabolic inflammation perturbs the insulin signaling pathway and leads to insulin resistance. Researchers have identified several pathways that link the impairment of insulin resistance through obesity-induced inflammation like activation of Nuclear factor kappa B (NF-κB), suppressor of cytokine signaling (SOCS) proteins, c-Jun-N-terminal Kinase (JNK), Wntless-related integration site (Wnt), and Toll-like receptor (TLR) signaling pathways. In this review article, the published studies have been reviewed to identify the potential and influential role of different signaling pathways in the pathogenesis of obesity-induced metabolic inflammation and insulin resistance along with the discussion on potential therapeutic strategies. Therapies targeting these signaling pathways show improvements in metabolic diseases associated with obesity, but require further testing and confirmation through clinical trials.

1. Introduction

The need for nutrition begins at fertilization, continues through life, and ends at death [1]. A balanced diet is composed of adequate amounts of essential nutrients, fats, carbohydrates, and proteins required by body to obtain energy, but any disturbance in a balanced diet leads to the development of diseases. For instance, nutrient deficiencies in humans can cause a variety of diseases, including protein energy malnutrition, anemia, pellagra, beriberi, and scurvy [2]. Conversely, excessive nutrient consumption and their accumulation in adipocytes can result in obesity [3], which increases the risk of developing chronic diseases such as type 2 diabetes, inflammation, cardiovascular disease, cancer, and hypertension [4]. Around 1.9 billion people worldwide were estimated to be obese in 2016, and virtually every country has seen this

number increase [5]. According to the World Obesity Atlas 2022, it is confirmed that by 2030, 1 in 5 women and 1 in 7 men will be affected by obesity (BMI ≥ 30 kg/m²), which is equivalent to 1 billion people globally [6].

As inflammation and insulin resistance are commonly associated with obesity, they play an important role in impairing human health by fluctuating glucose and lipid metabolism in skeletal muscle, liver, and adipose tissue [7]. In normal individuals, the insulin signaling pathways is activated and participates in regulating glucose production in the liver. These pathways also trigger the uptake of glucose into fat and muscle cells and thus help maintain glucose homeostasis in the body. But a decrease in the activity of the insulin signaling pathways is thought to be a major cause of the development of low-grade inflammation in obesity and insulin resistance [8]. The development of low-grade

Abbreviations: NF-κB, Nuclear factor kappa B; SOCS, Suppressor of cytokine signaling; JNK, c-Jun-N-terminal Kinase; Wnt, Wntless-related integration site; TLR, Toll-like receptor; TNF-α, Tumor necrosis factor-α; IL, Interleukin; IRS, Insulin receptor substrate; PPAR, Peroxisome proliferator-activated receptor; MyD88, Myeloid differentiation factor-88.

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Pectin-based drug delivery systems: Enhancing site-specific therapeutic efficacy and bioavailability

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ABSTRACT

The targeted delivery of therapeutic agents to specific anatomical sites in the body is pivotal for optimizing drug efficacy, safety, and patient compliance. While oral and transdermal routes are preferred methods for drug delivery, they encounter obstacles such as acidic pH, the presence of gastrointestinal microflora, and low skin permeation rates. The global scientific community continues to face the ongoing challenge of selecting a polymer with robust stability that can effectively deliver drugs to specific sites. Numerous polymers have been acknowledged for their ability to prevent drug degradation, among which pectin stands out as a naturally occurring biocompatible polymer with significant potential. In recent decades, scientists have increasingly prioritized the development of pectin-based delivery systems to achieve prolonged drug release, highlighting the significance of pectin in biomedical research. The encapsulation of drugs within pectin enhances drug bioavailability and stability, thereby aiding in reducing the risk of drug degradation. This review aims to highlight the beneficial effects of newly developed pectin-based delivery systems in improving drug delivery by enhancing bioavailability. Additionally, it includes discussion on challenges and considerations regarding future perspectives for developing more efficient formulations to align with regulatory guidelines and ensure patient compliance.

1. Introduction

Drug delivery entails delivering therapeutic agent to its designated location, enhancing therapeutic efficacy to ameliorate the disease condition. The effectiveness and precision of successful treatment, aided by efficient drug delivery, are pivotal in predicting treatment success (Tewabe et al., 2021). Physicians in clinical practice have historically relied on traditional methods such as oral and transdermal medications because they are safe, convenient, and effective. However, these methods of drug administration have posed challenges for physicians since ancient times, as the drugs face various barriers that hinder absorption, bioavailability, solubility, and penetration rate, ultimately impeding delivery to the target site (Bhutani et al., 2021). This unsafe conveyance of drugs through the traditional oral and transdermal route diminishes the clinical therapeutic outcomes, prompting researchers to seek alternatives to surmount these barriers and effectively deliver drugs to their intended sites.

In response to these challenges, researchers around the world have

been actively seeking alternatives that not only aid in directing drugs to their intended sites but also amplify their therapeutic effects in treating diseases (Patra et al., 2018; Lushchak et al., 2020). For instance, researchers have developed nanodelivery systems to efficiently target drug release by encapsulating drugs within polymer matrices. These polymers protect the drugs, facilitating their passage through gastrointestinal barriers in oral delivery and enhancing penetration rates in transdermal delivery (Pires et al., 2023). Thus, polymers are crucial in ensuring the successful and precise delivery of drugs to their intended sites, improving therapeutic efficacy and minimizing side effects. Polymers present numerous benefits compared to inorganic carriers such as mesoporous silicon and metal nanoparticles for drug delivery purposes. On the other hand, although inorganic carriers may present distinctive physicochemical properties and significant drug loading capabilities, they also pose potential issues concerning biocompatibility, toxicity, and long-term safety (Ezike et al., 2023).

Natural polymers emerge as an encouraging and adaptable approach for drug delivery, amalgamating biocompatibility, customizable attributes, and physiological alignment to address the dynamic requirements

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Poster Presentation Certificate

IDF WORLD DIABETES CONGRESS 2025

On behalf of the International Diabetes Federation and the Congress Organising Committee, we would like to thank you for participating in the IDF Congress 2025 World Diabetes Congress in Bangkok, from 7th to 10th April 2025. We confirm that the below mentioned poster was presented at the congress.

Poster Title:

Polymeric Nanoformulation of Acarbose Enhances Anti-Diabetic Efficacy and Reduces Oxidative Stress in HFD/STZ Rats

Presenting Author:

Mr Sourbh Suren Garg

All Authors:

GARG, S.(1)*;Gupta, J.(1);

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CERTIFICATE OF RECOGNITION

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"Acarbose-Encapsulated Guar Gum Nanoformulation: A Promising Strategy for Type 2 Diabetes Management"

At the

"9th International Conference on Material Science and Engineering"
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A handwritten signature in black ink, appearing to read "MS" followed by a stylized flourish.

MARCIN H. STRUSZCZYK
Instytut Technologii Bezpieczeństwa
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**International Conference on Advances in Biological Sciences
for Sustainable Development**

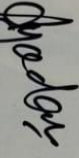
(ICABSSD-2024)

1-2 March, 2024

Department of Zoology, Central University of Jammu

Certificate

This is to certify that Prof./Dr./Ms./Mr. SOUBH SUREN GARD has
delivered a ~~Keynote talk / Invited talk~~ / ~~Chair~~ a session / ~~Co-chaired a session~~ / ~~Oral presentation~~ / ~~Poster presentation~~ /
Participated / ~~Volunteer~~ in the "International Conference on Advances in Biological Sciences for Sustainable Development
(ICABSSD-2024)" at the Department of Zoology, Central University of Jammu, Jammu and Kashmir, India.


Dr. Ashok Kumar Yadav
(Convenor)


Dr. Dhahananjay Kumar
(Co-Convenor)


Dr. Shvetambri Jasrotia
(Organizing Secretary)



INCD 2024



6th International Conference on Nutraceuticals & Chronic Diseases
&

10th Meeting of Society for Translational Cancer Research
"Nutraceuticals: Basic Science to Clinical Applications"
February 22-24, 2024

Certificate of Participation

This is to certify that Savithi Suman Garg.....has participated in INCD-2024
as a Delegate/Invited Speaker/Chariperson and presented a talk/paper (oral/Poster) entitled
"Formulation, development, and manufacturing of a Diabetes".
Panjab University, Chandigarh

Navneet Agnihotri
Prof. Navneet Agnihotri
Organising Secretary

Rajat Sandhir
Prof. Rajat Sandhir
Co-Organising Secretary