Exploration of therapeutic effect of 4-phenylbutyric acid against diabetic complications

Thesis submitted in partial fulfillment of the requirements of the degree of
MASTER OF PHARMACY

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CERTIFICATE OF APPROVAL

This is to certify that the thesis entitled "Exploration of therapeutic effect of 4-phenylbutyric acid against diabetic complications" has been carried out by Mr.Vishwakarma Vishal Phulchand under the supervision of Prof. Saikat Dewanjee, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, 700032. He has incorporated his findings into this thesis of the same title, being submitted by his, in partial fulfillment of the requirements for the degree of "Master of Pharmacy" of this university. He has pursued this work independently with proper attention to my entire satisfaction.

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DEDICATED TO MY PARENTS

Mr. Phulchand Vishwakarma & Mrs. Seema

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Chapter 1

Introduction

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1. Introduction

Diabetes mellitus (DM) is a long-term metabolic disease marked by high blood glucose levels. This disorder develops when the body either produces insufficient insulin (a hormone that controls blood sugar) or when cells stop responding to insulin (Chinyere et al., 2022). Diabetes is classified into four types. Type 1 diabetes (T1DM) occurs when the immune system targets and kills the pancreatic β-cells that produce insulin, hence T1DM is considered an autoimmune condition. On the other hand, a combination of lifestyle factors, genetic predisposition, and insulin resistance usually leads to the development of type 2 diabetes mellitus (T2DM). Regardless of type, DM has profound effects on the body's many organs and systems (Yan et al., 2024). People with DM are becoming more and more common., and monetary pressure on any healthcare system. In 2017, 5.0 million individuals worldwide, aged 20 to 99, lost their lives due to diabetes-related causes (Saeedi et al., 2019). In 2019, 463 million individuals worldwide, or 9.3% of the total population, had diabetes. By 2045, the disease is predicted to affect 700 million people worldwide, or 10.9% of the total population (Saeedi et al., 2019). T1DM, which results from the degeneration of pancreatic β-cells, affects 5–10% of diabetic patients. (Pinchevsky et al., 2020). A substantial risk of developing diabetic ketoacidosis (DKA), diabetic coma, and possibly mortality exists in many T1DM patients (Levy-Marchal et al., 2001). Immune-mediated etiology has been proposed as the cause and the name implies, that latent autoimmune diabetes in adults (LADA) is a T1DM that manifests later in life (Leslie et al., 2006). 90-95% of diabetic patients are suffering from T2DM (American Diabetes Association, 2017). This kind of diabetes mellitus is caused by a progressive decrease in insulin secretion, which may be partially attributed to obesity, a deterioration in pancreatic β-cell activity, and ultimately hyperglycemia (Stumvoll et al., 2005). Insulin resistance, which can vary from a relative deficiency to a total insulin secretory failure is developed by patients with T1DM moreover interplay of genes and environment is most likely linked to the etiology of T1DM (Romao and Roth, 2008). People with high blood pressure, high body mass index (BMI), and other cardiovascular risk factors are often observed to have T2DM, therefore it is regarded as a "disease of lifestyle". Various pharmacotherapy, such as combinations of oral and frequently insulin-based antihyperglycemic drugs, antihypertensives, and lipid-lowering medicines is used in the management of T2DM (Ohanson and Pretorius, 2023). Gestational diabetes mellitus (GDM) is

another form of diabetes that only manifests itself during pregnancy. Between 5 and 15% of pregnant women have GDM, with regional and racial variations, (WHO, 2022). Multifarious factors including genetic defects, pancreatic obstruction, surgery, and organ transplantation contribute to the onset of this type of diabetes (Alam et al., 2021). In the case of 40–60%, women having GDM can develop DM after 5–10 years of pregnancy. Impaired glucose tolerance is potent to be expressed as T2DM whereas uncontrolled diabetes is the potential threat for the onset of other diseases like cardiovascular disease (CVD), blindness, renal failure, neurological disorder, the imbalanced osmolality of blood, hypertension, peripheral neuropathy, and many other diseases [10–14Monogenic diabetes, which is often misdiagnosed as T1DM or T2DM is caused by a mutation in a single gene or a cluster of genes (Zhang et al., 2021).

1.1. Complications of DM

Exposure to diabetes for an extended period exposes many more issues. Diabetic retinopathy and diabetic nephropathy are brought on by persistently high blood sugar levels. Prolonged elevation in blood glucose levels may cause the enzyme myo-inositol oxygenase to become more active, which further encourages the breakdown of myo-inositol. Enzymatic degradation of Myo-inositol affects phosphatidylinositol synthases and Na+/K+ ATPase, two extremely significant components in the secondary signaling pathway (Yang et al., 2010). Therefore, increased blood glucose levels brought on by diabetes mellitus result in diabetic neuropathy, diabetic retinopathy, and diabetic cataracts. Uncontrolled diabetes damages the retina's blood supply by increasing the osmotic pressure in the retina's delicate vessels as a result of elevated blood glucose levels. leading to Vessels leaking or bursting under particular conditions. Collateral blood vessels emerge from the retina to compensate for the burst retinoid vessels, resulting in tissue scarring and vision impairment (Tarr et al., 2013; Antonetti et al., 2012). Diabetic nephropathy is a condition when proteins in the urine escape through the basement of glomerular capillaries due to uncontrolled diabetes, which damages the kidneys (Lim, 2014). Diabetes patients often experience ketoacidosis due to their continuous synthesis of ketone bodies (Ifebi and Onyiriuka,2013). Unlike diabetes mellitus (DM), which is characterized by insulin resistance, diabetic ketoacidosis (DKA) is characterized by insulin insufficiency. Furthermore, individuals with diabetes are more vulnerable to the damaging effects of free radicals, raising their risk of atherosclerosis, cardiovascular disease, and hypertension. Heart disease, coronary artery disease (CAD), and sudden cardiac death are more common in patients with diabetes. Patients with

diabetes have higher blood glucose levels, which set off the Maillard reaction and produced superoxide and oxidative stress. Several studies have shown that T2DM is associated with cognitive impairment, which affects intelligence, attention, memory, learning, and perception. T2DM also affects the level of magnesium in the blood. Lowered blood magnesium levels result from reduced tubular reabsorption of magnesium brought on by elevated blood glucose (Barbagallo and Dominguez, 2015).

1.2. Mechanisms

Diabetes-related vascular problems, particularly type 2 diabetes, are significantly influenced by oxidative stress (Asmat et al., 2016). Reactive oxygen species (ROS) include hydroperoxyl, superoxide, hydrogen peroxide, and hydroxyl radicals. Free radicals are active derivatives of the oxygen molecule (Jomova et al., 2023). Due to unpaired electrons in their outermost molecule, these hyperactive components can interact with and alter other biomolecules. They have the ability to oxidize nucleic acids, proteins, and lipids, producing harmful byproducts that cause tissue malfunction (Radi et al., 2018; Sies et al., 2017). Furthermore, they modify and even shatter the architecture of biological molecules (Sies et al., 2017). One well-known consequence of oxidative stress, which impacts gene expression and cell viability, is DNA breakage. In addition to their direct harmful effects, free radicals can also cause indirect damage to cells by triggering a range of stress-sensitive intracellular signaling pathways, including hexosamine pathways, PKC (protein kinase C), AGE (advanced glycation end product) interactions, JNK (c-Jun NH(2)-terminal kinase), and sorbitol synthesis (Yaribeygi et al., 2020). Among the many indicators of oxidative stress in diabetic patients are reactive hydroperoxides (ROOH), total cholesterol, and malondialdehyde (MDA). Numerous pieces of evidence link oxidative stress to type 1 and type 2 diabetes. Due to the production of free radicals, glucose oxidation, nonenzymatic protein glycation, and increased lipid peroxidation, oxidative stress causes damage to enzymes, increased insulin resistance, and impaired cellular machinery (Yaribeygi et al., 2020). The hydroxyl radicals cause apo-B monomers to cross-link, which causes apolipoproteins and lipids to form insoluble aggregates and cause oxidative damage in complications connected to diabetes. Oxidative stress in diabetes mellitus is primarily caused by mitochondria (O'Neal et al., 2016). During oxidative metabolism, which is a significant source of reactive oxygen species (ROS), water is formed by reducing a portion of the oxygen that is used, and the remaining oxygen is converted to oxygen-free radical(s). The production of ROS/RNS in response to

insulin, which is necessary for full physiological function, is one way that ROS/RNS regulates the insulin signaling pathway. Another way is that ROS/RNS negatively regulates insulin signaling, which aids in the development of insulin resistance and type 2 diabetes mellitus (American Diabetes Association, 2010).

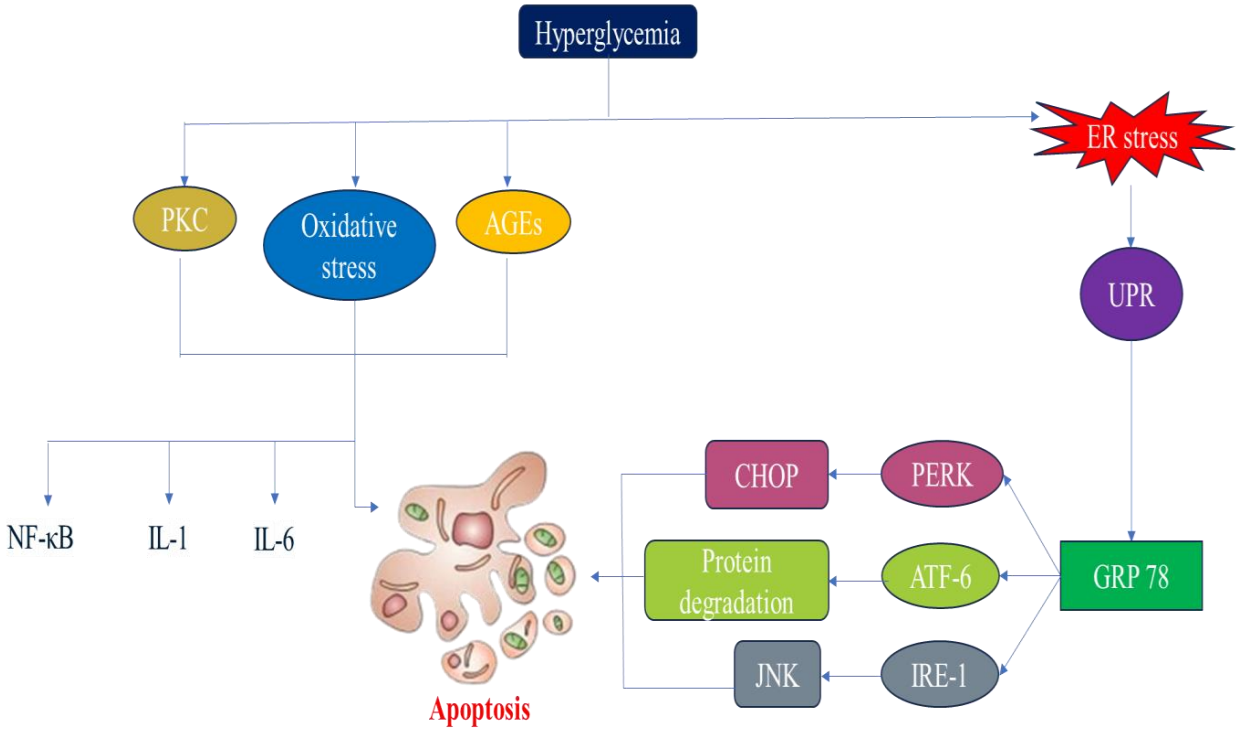

Figure 1. Mechanism of apoptosis caused by hyperglycemia

1.3. Conventional management of DM

One of the World Health Organization's top goals is the management of diabetes (Lovic et al., 2020). Diabetes problems put patients' health at risk and put a financial strain on societies (Zheng et al., 2018). Due to significant dietary and lifestyle modifications as well as rising industrialization, non-communicable diseases (NCDs), which pose a serious threat to human health, are affecting a growing percentage of the world's population. Currently accounting for 63% of all fatalities worldwide, NCDs are the leading cause of death (Alwan et al., 2010). Of these NCDs, 463 million people globally, or 9.3% of the world's population, suffered from diabetes in 2019. According to predictions, the illness will impact 700 million individuals

globally by 2045, or 10.9% of the world's population (Saeedi et al., 2019) earning the dubious distinction of being the diabetes capital of the world.

In the near future, DM is probably going to remain a major source of morbidity and mortality due to its rising occurrence around the globe. Since its origin, isolated or synthesized extracts have been the mainstay of modern medical science's approach to treating a wide range of diseases, including diabetes. However, continuous use of these traditional medications tends to be harmful and can result in unwanted consequences, drug dependency, etc. (Chaudhury et al., 2017) As a result, scholars and decision-makers are eager to explore established medical institutions for corrective action. The nation has long utilized the medical systems of Ayurveda, Unani, Siddha, and homeopathy. Specifically, Ayurveda uses natural materials for healing, such as minerals, metals, herbs, and other animal waste products. Since the beginning of human history, herbs have been primarily utilized in this field for medicinal purposes. In fact, a large portion of contemporary pharmacotherapy has its roots in herbal medicine. Products made from herbs have made a huge contribution to world health care. For a variety of reasons, they have drawn the attention of scientists and drug regulators throughout the last ten to twenty years and have grown in popularity (Galib et al., 2020). Many customers mistakenly think that because herbal medications are natural, they are safe, but this is a risky oversimplification. Herbal remedies can have negative effects, including interactions with prescription medications (Gouws and Hamman, 2020). As a result, not all herbal remedies are safe for all situations, and there is always a chance of herb-drug interactions, some of which are severe enough to endanger the health of the patient.

1.4. some commercially available agents used in treating diabetic complications along with their side effects

Table 1. **Commercially available agents used in treating diabetic complications**

Agent	Class	Indication	Side Effects	References
Angiotensin-converting enzyme (ACE) Inhibitors (e.g., **Lisinopril**)	ACE Inhibitor	Hypertension, Diabetic Nephropathy	Hyperkalemia, cough, hypotension, angioedema	ADA Standards of Medical Care, 2024

Angiotensin-II Receptor Blockers (ARBs) (e.g., **Losartan**)	ARB	Hypertension, Diabetic Nephropathy	Hyperkalemia, dizziness, fatigue, renal function decline	KDIGO 2020 Clinical Practice Guidelines
SGLT2-Inhibitor (e.g., **Empagliflozin**)	Sodium-Glucose Cotransporter-2 Inhibitor	Diabetic Nephropathy	Genital infections, dehydration, ketoacidosis, UTI	American Diabetes Association (ADA), 2023
Aldosterone Antagonists (e.g.,**Spironolact-one**)	Mineralocorticoid-Receptor Antagonist	Resistant Hypertension, Nephropathy	Hyperkalemia, gynecomastia, menstrual irregularities	JNC 8 Guidelines, 2014
Endothelin Receptor Antagonists (e.g., **Atrasentan**)	Endothelin Receptor Antagonist	Diabetic Nephropathy (Investigation Phase)	Fluid-retention, anemia, heart failure, hepatotoxicity	Heerspink etal.,The Lancet, 2019
Nonsteroidal Mineralocorticoid-Receptor Antagonists (e.g., **Finerenone**)	Mineralocorticoid-Receptor Antagonist	Diabetic Nephropathy	Hyperkalemia, hypotension, hyponatremia	Bakris et al., NEJM, 2020
GLP-1-Receptor Agonists (e.g., **Liraglutide**)	Glucagon-like peptide-1 Receptor Agonist	Type-2 Diabetes, Nephropathy (Secondary)	Nausea, vomiting, pancreatitis, diarrhea	Marso et al., NEJM, 2016

Dipeptidyl Peptidase-4 Inhibitors(DPP-)(e.g.,**Linagliptin**)	DPP-4 Inhibitor	Type-2 Diabetes, Diabetic Nephropathy (Secondary)	Upper respiratory infections, nasopharyngitis, headache	Groop et al., Lancet Diabetes Endocrinol, 2013
Statins (e.g., **Atorvastatin**)	HMG-CoA Reductase Inhibitor	Dyslipidemia, Cardiovascular risk reduction	Muscle pain, liver enzyme elevation, rhabdomyolysis	Ridker et al., JAMA, 2017

While current agents like ACE inhibitors, ARBs, and SGLT2 inhibitors provide some protection against the progression of diabetic nephropathy, they do not completely halt the progression of kidney damage. Many patients continue to experience a decline in kidney function despite optimal use of these therapies, highlighting the need for more effective treatments. The side effects associated with existing treatments can limit their use in certain patient populations. For example, ACE inhibitors and ARBs can cause hyperkalemia and hypotension, which are particularly concerning in patients with advanced kidney disease. Similarly, SGLT2 inhibitors are associated with a risk of genital infections and dehydration, which may not be well-tolerated by all patients. Despite the use of current therapies, patients with diabetic nephropathy remain at high risk for cardiovascular events and end-stage renal disease. This residual risk underscores the need for therapies that can address both kidney and cardiovascular outcomes more effectively. Diabetic nephropathy involves multiple pathophysiological pathways, including inflammation, oxidative stress, fibrosis, and endothelial dysfunction. Current therapies mainly target the renin-angiotensin-aldosterone system (RAAS) or glucose reabsorption in the kidney, leaving other pathways relatively unaddressed. This complexity necessitates the development of novel agents that can target these additional mechanisms. Research has identified new molecular targets, such as the endothelin system and mineralocorticoid receptors, that play a role in the progression of diabetic nephropathy. The success of drugs like finerenone, a nonsteroidal mineralocorticoid receptor antagonist, in clinical trials indicates that targeting these novel pathways may provide additional therapeutic benefits beyond what is achievable with current treatments. There is a growing recognition of the need for personalized approaches in treating diabetic nephropathy,

given the variability in disease progression and response to treatment among patients. Novel strategies could involve biomarkers that help tailor treatment to the individual patient's risk profile and disease characteristics, thereby improving outcomes. These factors collectively drive the ongoing search for new, more effective, and safer therapeutic strategies in the management of diabetic nephropathy.

1.5. Search for novel strategies

The endoplasmic reticulum (ER) is a crucial intracellular organelle that is frequently referred to as a factory for folding proteins. Lipid synthesis, glucose metabolism, and calcium storage all take place in the ER. Unfolded proteins can accumulate as a result of a number of clinical circumstances, such as sepsis, burns, trauma, and ischemia. These events can change the ER's homeostasis and result in ER stress (Khan et al., 2015). The function, adaptability, or death of stress cells have been shown to be strongly impacted by the severity and length of ER stress (Yoshino et al., 2017). A key player in controlling the unfolded protein response (UPR) signaling network is glucose-regulated protein 78 (GRP78), a marker for ER stress (Cao et al., 2020). In non-stressful circumstances, GRP78 attaches itself to ER transmembrane proteins, such as membrane-embedded proteins RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring protein 1 (IRE1), and activating transcription factor 6 (ATF6). GRP78 dissociates from these transmembrane proteins in response to ER stress, which causes them to become activated and sets off three different forms of UPR. This, in turn, regulates the production of particular transcription factors and UPR downstream pathways (Gardner et al., 2013). As a result of the numerous studies that have been done recently on the regulation of the ERS pathway, ERS targeting has emerged as a significant new therapy option for DKD. Drugs that specifically regulate endoplasmic reticulum calcium balance, UPR response, and protein folding are examples of ERS-related medications (Marciniak et al., 2022). One of the few ERS inhibitors that has been approved by the US Food and Drug Administration is 4-phenylbutyrate (4-PBA). As an ammonia scavenger, its primary application is in the treatment of urea cycle diseases (Kolb et al., 2015). Since ERS plays a crucial role in the pathophysiology of diabetes, recent research has shown that 4-PBA can treat severe sepsis and septic shock in rat models and can considerably improve essential organ function and overall treatment results without any adverse effects (Liu et al., 2016). Thus, preventing or reducing ERS in β-cells may be a unique approach

to diabetes treatment and prevention. Furthermore, in primary rat islets, 4-PBA has been demonstrated to enhance the inhibition of palmitate-induced glucose-stimulated insulin secretion (GSIS). It accomplishes this by lowering ER stress in obese animals to bring blood glucose and insulin sensitivity back to normal (Zhou et al., 2021). Remarkably, 4-PBA has also been demonstrated to alleviate β-cell dysfunction and insulin resistance in people that were brought on by a sustained increase in free fatty acid levels. 4-PBA-dependent ERS decrease was the cause of these effects (Xiao et al., 2011). 4-PBA functions as an ER stress inhibitor by eradicating intracellular UPR and regulating multiple essential proteins, such as BIP, PERK, ATF6, IRE1, and CHOP. Consequently, 4-PBA may be regarded as a novel diabetic treatment. We also demonstrate in our work that 4-PBA reduces ER stress. Nevertheless, additional research is needed to determine how 4-PBA affects β-cell activity.

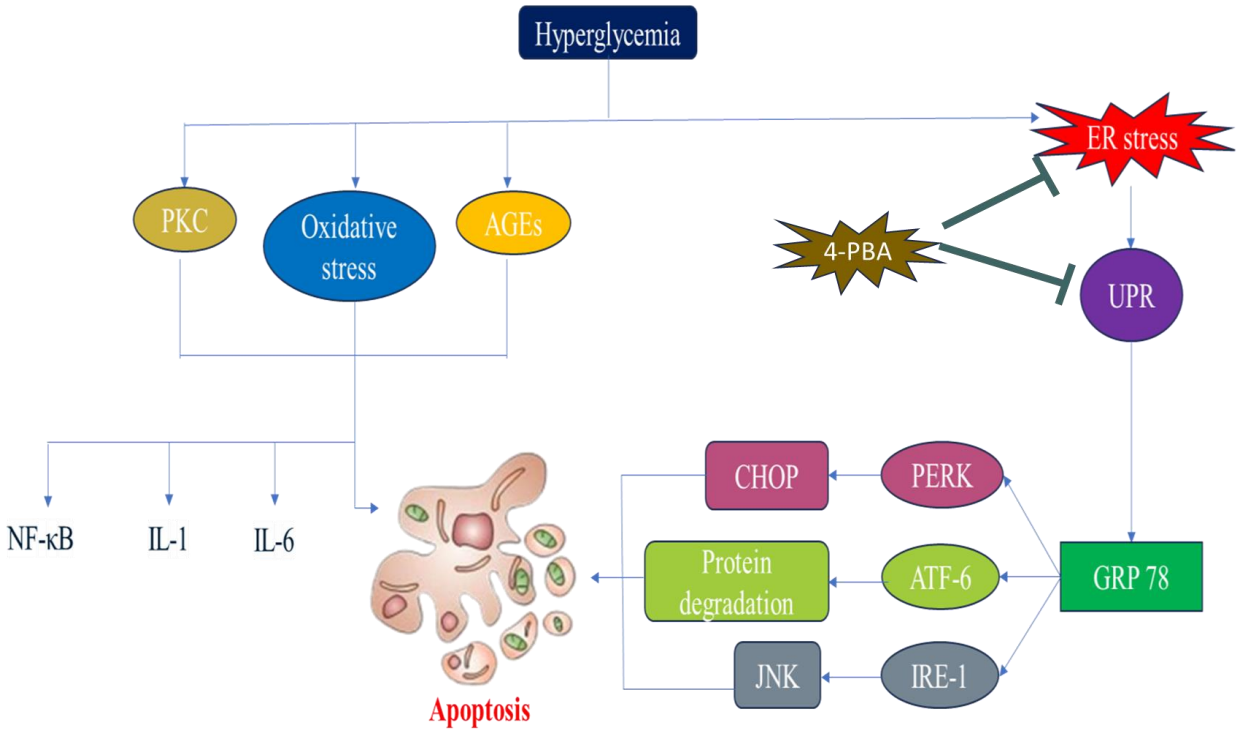

Figure 2. Protective mechanism of 4-PBA apoptosis caused by hyperglycemia

1.6. Advantage of 4-PBA on diabetic nephropathy

Postbiotics are defined as follows: (i) soluble factors secreted by live bacteria, or released after bacterial lysis, such as organic acids, peptides, teichoic acids, polysaccharides, cell-surface proteins, and peptidoglycan-derived muropeptides; (ii) non-viable metabolites produced by microorganisms that exert biological effects on the hosts; and (iii) compounds produced by microorganisms that are released from food ingredients or microbial constituents, including non-viable cells, that, when administered in sufficient amounts, promote health and wellbeing. When given to consumers, these compounds either directly or indirectly mediate advantageous biological processes (Gurunathan et al., 2023).Postbiotics, defined as "preparations of inanimate microorganisms and/or their components that confer a health benefit to the host," are created by a variety of bacterial and fungal species that are naturally present in fermented foods like yoghurt, sauerkraut, pickled vegetables, and kombucha. These species include *Lactobacillus, Bifidobacterium, Streptococcus, Eubacterium,* and *Saccharomyces*. (Barros et al., 2021; Amores-Arrocha et al., 2018). A number of commercial postbiotics are available as supplements or in food matrices; they are mostly used to treat gastrointestinal or immune-related illnesses (Cuevas et al., 2020). Typically, postbiotics include extra medicinal and wellness-enhancing ingredients. Healthy bacteria are maintained by metabolites, which also lessen the likelihood of dangerous microbes existing.Large amounts of butyrate and Short Chain Fatty Acids (SCFAs) are produced when many microorganisms in the gastrointestinal tract catabolise indigestible carbohydrates (Fong et al., 2020). 4-PBAs are categorised as SCFAs; they lower lipid levels, shield cells from oxidative damage, stop the synthesis of fatty acids, and stop inflammatory illnesses (Torino et al., 2015).Reactive oxygen species (ROS) have the ability to alter the properties of proteins and lipids. This can ultimately result in cellular dysfunction and long-term problems like diabetes and its aftereffects, microvascular disease, and cardiovascular effects. Living things can employ enzymatic or non-enzymatic defenses, such as natural antioxidants (vitamins C and E), to quench ROS in order to combat ROS followed by ERS (Giacco et al., 2010; Abdali et al., 2015). A few of the notable qualities of postbiotics are as follows: (i) they are generally safe; (ii) they are well-tolerated and associated with a lower risk of negative effects in susceptible individuals (Gurunathan et al., 2023); (iii) they are quite safe (Scarpellini et al., 2021); (iv) they do not pose a risk of spreading antibiotic-resistant genes to pathogenic or commensal bacteria; (v) their effectiveness is independent of cell viability, leading to increased stability and shelf life. (vi)

They exhibit interesting technological features, such as the rheological properties of exopolysaccharides (EPSs) used as stabilisers in the food industry (Nataraj et al., 2020) (vii) They have a wide range of health-promoting effects; and (viii) They include antifungal and antibacterial agents (Cabello-Olmo et al., 2021).

Chapter 2

Literature Review

Content

2. Literature review

4-Phenylbutyrate (4-PBA) is a naturally occurring aromatic short-chain fatty acid that is derived from butyric acid through fermentation by intestinal bacteria. Butyrate has a wide range of effects at the intestinal level, such as reducing mucosal inflammation, controlling transepithelial fluid flow, enhancing oxidative state, and preventing colon cancer. Furthermore, a growing body of research indicates that butyric acid can prevent or inhibit different forms of cancer by causing cancer cell growth arrest and apoptosis. In a similar vein, phenylbutyrate exhibits potentially beneficial effects on a wide range of pathologies, such as urea cycle disorders, cancer, neuropathies, diabetes, hemoglobinopathies, and inherited metabolic syndromes. 4-PBA has distinct methods through which it achieves various effects. Some of them are involved in the control of gene expression, functioning as inhibitors of histone deacetylase; others are involved in the ability to rescue proteins from conformational abnormalities, acting as chemical chaperones; still, others are involved in its metabolic feature, which permits the excretion of harmful ammonia, thereby functioning as scavengers of ammonia. The term "butyrate paradox" has been proposed because phenylbutyrate can have different effects depending on the kind of cell. These findings point to a wide range of beneficial outcomes elicited by PBA with significant therapeutic potential. The three primary branches of PBA's molecular action are the topic of our review, which focuses on the cellular and systemic effects of PBA treatment (Kusaczuk et al., 2015).

2.1. Scientifically explored pharmacological activities of 4-PBA

2.1.1. Role in urea cycle disorder

To further clarify the role of ER stress in the phenotypic shift in HBZY-1 cells caused by uric acid, 4-phenyl butyric acid, an ER stress inhibitor, was employed. It is likely that uric acid damages the kidneys through two main methods. First, endothelial dysfunction and inflammation are brought on by hyperuricemia (Karbowskan et al., 2009). Prostaglandin E2 and monocyte chemoattractant protein 1, which are implicated in the inflammatory response, are produced by renal epithelial cells exposed to UA crystals (Khan, 2004). Second, UA can raise renin expression in rats and hyperuricemia can confound glomerular hemodynamics (Nakagawa et al., 2003) also Interstitial fibrosis and tubular atrophy are related to ER stress (Chiang et al., 2011). Interestingly, the current investigation revealed that UA dramatically increased the mRNA and protein expressions of GRP78 and PDI in HBZY-1 cells in a manner that was dependent on both

concentration and time, indicating that ER stress plays a role in the harm that HBZY-1 cells sustain. Clinically authorized as an ammonia scavenger in children with urea cycle abnormalities, 4-phenyl butyric acid is an aromatic short-chain fatty acid with chaperone-like properties (Singh et al., 2006). Significantly, 4-PBA functions as a chemical chaperone in the ER and can start the stabilization of peptide structures, which enhances the luminal folding ability and aberrant protein trafficking (Inagi et al., 2010). 4-PBA demonstrates the advantageous effects of ER stress modulators by saving mutant nephrin linked to misfolding and mislocalization (Li et al., 2013). In the current investigation, we found that in rat renal mesangial cells cultivated in UA, 4-PBA administration significantly decreased the expression of α-SMA and relieved FN expression. Thus, glomerular damage caused by UA may be significantly influenced by ER stress. These findings demonstrate how 4-PBA protects glomerular mesangial cells from damage caused by UA.

2.1.2. Role in osteoarthritis

One possible treatment for osteoarthritis is to improve protein folding in the ER. This could be accomplished by administering small-molecule chemical chaperones, such as 4-phenyl butyric acid (4-PBA). 4-PBA was shown to efficiently diffuse into cartilage explant cultures and lessen excessive ER stress in chondrocytes in a dose-dependent manner. (Rellmann et al., 2019). Others have shown that cruciate ligament transection administered intragastrically in a rat model, 4-PBA exhibits preventative qualities against the onset of osteoarthritis. 4-animals after administration of 4-PBA resulted in reduced apoptotic cells, cytokine production, and tissue damage. This was followed by the downregulation of apoptotic marker proteins and ER stress. (Tang and others, 2018).

2.1.3. Role in Parkinson’s disease

According to a study, 4-PBA, a chemical chaperone, encourages proper protein folding (Kudo et al., 2008; Engin et al., 2010; Rajan et al., 2011). According to reports, 4-PBA or its derivatives reduce the cell death brought on by the accumulation of Pael-R and encourage the proper folding of unfolded Pael-R (Mimori et al., 2012). Additionally, ER stress-induced neuronal death is inhibited by the molecular chaperone inducer Bip inducer X (BIX). These studies suggest that Parkinson's disease (PD) and other neurodegenerative illnesses resulting from ER stress may benefit from the use of 4- PBA and accelerate proper protein folding. (Omura et al., 2013).

2.1.4. Role in vasculitis peripheral neuropathy

Vascular peripheral neuropathy (VPN) is characterized by acute to subacute sensory and motor difficulties that occur from inflammatory nerve blood vessel obliteration, causing ischaemic injury to peripheral nerves. (Kwak et al., 2009). Recent animal studies suggest that preserving or restoring ER activity could be therapeutic for neuropathic pain associated with particular peripheral neuropathies, such as diabetic peripheral neuropathy induced by streptozotocin and formalin-induced peripheral inflammatory pain (Inceoglu et al. 2015; Zhou et al. 2017). 4-PBA has been demonstrated to concurrently reduce the effector molecules eIF2α, ATF4, and CHOP in ER stress signaling and the activation of NF-κB in neuroinflammation. Moreover, 4-PBA simultaneously prevented PERK, IRE1, and ATF6—upstream ER stress receptors—from activating. The molecular findings indicated that ER stress and NF-κB-mediated neuroinflammation were both incorporated in this VPN model. More importantly, the behavior study demonstrated that the neuropathic pain and motor impairment associated with VPN were alleviated by 4-PBA-induced ER stress inhibition. (Chen et al.,2019)

2.1.5. Role in acute lung injury

Lipopolysaccharide (LPS) has been identified as a significant contributing factor to pulmonary damage (Opal et al., 1999). The common symptoms of acute inflammatory reactions in the lung are brought on by the LPS challenge, which causes serous fluids to flow into lung tissues. It is unknown, how inflammation contributes to the development of ALI. It is commonly understood that cells respond to disruptions in ER homeostasis through an adaptation process known as ER stress. The lung's expressions of GRP78, CHOP, and Caspase-12 were elevated by LPS. These expressions were then reduced by 4-PBA treatment, which also prevented the phosphorylation of the ER stress sensor proteins ATF6, XBP1, and PERK (Ma et al., 2020). The progression of LPS-induced acute lung injury has been demonstrated to be significantly influenced by ER stress, offering more proof of the pro-inflammatory nature of ER stress. Thus, it has been determined that 4-PBA prevented LPS-induced lung damage by reducing inflammation and ER stress and that autophagy mediated by ER stress has cytoprotective effects on lung damage caused by LPS. These results could improve our comprehension of the cellular pathophysiological changes in the lung after acute lung injury (ALI) (Zeng et al., 2022) and this finding seems to be very crucial in applying the concept to the treatment of diabetic nephropathy.

2.1.6. Role in human gingival fibrosis

As an ER stress antagonist, 4PBA has been shown in a study to reduce Advanced Oxidative Protein Products (AOPP) levels in gingival fibroblasts (Assaggaf et al., 2015). Numerous investigators have noted fibrosis indicators such as connective tissue growth factor (CTGF), transforming growth factor β (TGFβ), and α smooth muscle actin (αSMA) (Chen et al., 2016; Yang et al., 2015). The production of oxidative stress and the drug's tendency to promote Endoplasmic Reticulum (ER) stress in renal tubular cells are two noteworthy findings in cyclosporine-triggered renal fibrosis (O'Connell et al., 2012). Increased intracellular calcium, increased oxidative stress, and occasional medication use are all possible causes of endoplasmic reticulum stress (Supraja et al., 2016). In human gingival fibroblasts treated with cyclosporine, we also noticed elevated intracellular and oxidative stress (increased advanced oxidation protein product) (Tang et al., 2015). Cyclosporine causes ER stress in tubular cells, which in turn triggers the epithelial-mesenchymal transition (EMT) in renal fibrosis. Therefore, both variables (intracellular calcium and ROS) are also good candidates for causing ER stress in gingival fibroblasts.ER stress is linked to numerous illnesses, such as periodontitis, an inflammation of the tooth-supporting structures brought on by biofilms. individuals with periodontitis have higher levels of unfolded protein response (UPR) associated gene expression than individuals with gingivitis (Domon et al., 2009). Devastating diseases can result from ER stress in a cell, which can cause apoptosis, fibrosis, and the epithelial-mesenchymal transition (EMT). The cross-talk between the ER and mitochondria, which results in the overexpression of pro-apoptotic proteins, is what causes this ER stress-induced apoptosis (Senft & Ronai, 2015).

2.1.7. Role in Alzheimer’s disease

According to numerous publications, 4-PBA can function as a chemical chaperone by stopping misfolded proteins from aggregating (Rubenstein and Zeitlin, 2000; Kubota et al., 2006). Histone acetyltransferases (HATs) and histone deacetylases (HDACs) catalyze the acetylation and deacetylation of histones, respectively. According to a competing model, 4-PBA has an HDAC (Histone deacetylase) inhibitor activity and may therefore regulate the expression of various neuronal genes during neurodegenerative disorders that involve aberrant histone acetylation (Ricobaraza et al., 2009; Chuang et al., 2009; Shein and Shohami, 2011). DNA methylation and the equilibrium between HATs and HDACs control the expression of epigenetic genes. Histones that have been partially loosened by acetylation facilitate transcription factors' ability to bind to

genes with ease. This results in the expression of several genes as well as epigenetic transcription, which controls cellular homeostasis. HDACs are hypothesized to rectify constitutively overexpressed related proteins found in many cancer types by restoring aberrant gene expression to normal. HDAC inhibitors have therefore been created as anticancer medications. Several other outstanding reviews (Parbin et al., 2014; Cruz and Matushasky, 2012; Dokmanovic et al., 2007) have already provided a summary of this research. In contrast to cancer, neurodegenerative diseases are characterized by decreased protein turnover and disrupted homeostasis. However, aberrant gene expression is present in both neurodegenerative illnesses and malignancies. Histones with reduced acetylation are generally thought to be the cause or at least a contributing factor in neurodegenerative disorders. HDAC inhibitors are therefore now being considered as potential therapeutic medicines to help restore the proper balance of gene expression (Das et al., 2016).

2.1.8. Role in pulmonary arterial hypertension

ER stress—caused by unfolded and/or misfolded proteins being packaged in the ER—has been demonstrated in recent research to be a significant factor in the development of PAH (Sakao et al., 2009; Fijalkowska et al., 2010). When there is mild ER stress, the unfolded protein response (UPR) increases the synthesis of ER chaperones such as GRP78 and GRP94, which optimize protein folding and maintain proper Ca2+ and ATP levels. This is an attempt to decrease the number of misfolded proteins (Yeager et al., 2012). Nevertheless, chronic UPR activation causes apoptosis and cellular dysfunction, which encourage pulmonary arterial remodeling (Dromparis et al., 2013). Three distinct signaling mechanisms, including activating transcription factor-6 (ATF6), inositol requiring enzyme-1 (IRE1), or PKR-like ER kinase (PERK) in the ER membrane, cause UPR-induced cellular death. One novel approach to treating PAH was presented in a study conducted by Evangelos D. Michelakis and colleagues. They found that ATF6 activation may result in the proliferation of smooth cells in the pulmonary vascular system and that pharmacological chaperone inhibition of ATF6 may alleviate symptoms of PAH (Sutendra et al., 2011). 4-PBA is an oral, low-toxicity substance that provides protection against a range of unpleasant stimuli. Consequently, it has been suggested as a treatment for cancer, cystic fibrosis, and sickle cell disease (Kolb et al., 2015). Crucially, 4-PBA functions as a chemical chaperone that inhibits ER stress by attenuating cell damage, strengthening luminal folding capability, and stabilizing peptide structures (Frump et al., 2013). A recent study found

that 4-PBA can dramatically lower the expression of effector molecules in the ATF6 branch of ER stress signaling. GRP78 is a precursor to ER stress and a reliable marker of the initiation of the ER stress signaling cascade. In line with the suppression of GRP78 expression, ER stress is inhibited. In addition to ATF6 suppression, PBA-treated groups also showed decreased expression of CHOP, a command executor in the ER stress pathway. 4-PBA has been shown in this study to be able to dramatically lower effector molecule expression in the ATF6 branch of ER stress signaling. GRP78 is a precursor to ER stress and a reliable marker of the initiation of the ER stress signaling cascade. In line with the suppression of GRP78 expression, ER stress is inhibited. In addition to suppressing ATF6, PBA-treated groups also showed decreased expression of CHOP, a command executor in the ER stress pathway.

2.1.9. Role in atherosclerosis:

Vulnerable atherosclerotic plaques dramatically raise the risk of acute coronary syndromes, which are defined as rapid plaque ruptures resulting in potentially fatal coronary thrombosis (Falk et al., 2013; Lutgens et al., 2003). Large necrotic cores, increased inflammation, and thin collagen layers are common characteristics of these plaque rupture sites, all of which add to their lethal nature (Dong et al., 2015). It has been demonstrated that ER stress has a role in the development and advancement of atherosclerosis. For example, ER stress brought on by lipotoxicity causes the release of chemokines and pro-inflammatory cytokines, which worsen endothelial function and make it easier for monocytes to be recruited (Hotamisligil, 2010). Multiple processes have been identified as links between ER stress and plaque destabilization. First, smooth muscle cells within the fibrous cap undergo apoptosis as a result of ER stress, which thins the cap and makes it more prone to rupture (Tabas and Ron, 2011). Furthermore, atorvastatin was shown to significantly decrease ER stress in hyperhomocysteinemic mice, hence improving the stability of atherosclerotic plaques (Jia et al., 2016). Moreover, by inducing cellular apoptosis and activating ER stress pathways, cold stress has been shown to exacerbate the instability of atherosclerotic plaques (Dia et al., 2014). This work demonstrates that the chemical chaperone 4-PBA can greatly increase atherosclerotic plaque stability (Zhu et al., 2024).

Chapter 3

Aim & Objective

Content

3. Aim and Objective

3.1. Aim

To investigate the therapeutic potential of 4-phenylbutyric acid (4-PBA) in ameliorating complications associated with Streptozotocin (STZ)-induced diabetes in a Wistar rat model, with a focus on its effects on diabetic nephropathy, oxidative stress, and metabolic disturbances.

3.2. Objectives

3.2.1. To evaluate the impact of 4-PBA on body weight and kidney weight in STZ-induced diabetic rats, assessing its potential to mitigate diabetes-related weight loss and renal hypertrophy.

3.2.2. To assess the effect of 4-PBA on blood biochemical parameters, including liver function markers (ALT, AST), kidney function markers (BUN, urea, creatinine), inflammatory markers (CRP), and glycaemic control (HbA1c), in STZ-induced diabetic rats.

3.2.3. To determine the influence of 4-PBA on serum lipid profile, specifically measuring HDL, LDL, and triglyceride levels, and its ability to normalize lipid metabolism in diabetic conditions.

3.2.4. To investigate the role of 4-PBA in modulating oxidative stress parameters, including levels of superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione (GSH), in kidney tissues of STZ-induced diabetic rats.

3.2.5. To examine the histopathological changes in kidney tissues of STZ-induced diabetic rats following 4-PBA treatment, evaluating its efficacy in reducing glomerular hypertrophy, mesangial matrix expansion, and glomerulosclerosis.

3.2.6. To explore the overall potential of 4-PBA as a therapeutic agent in preventing or reducing the progression of diabetic nephropathy and other diabetes-related complications in a rat model.

Chapter 4

Materials and Methods

Contents

4. Materials and methods:

4.1. Chemicals

Reagents. Streptozotocin (STZ) and 4-Phenyl butyric acid (4-PBA) were procured from Sigma Aldrich Co (St Louis, U.S.A.). Plasma insulin, blood glucose, superoxide dismutase (SOD), and Glutathione (GSH) assay kits were procured from Abcam (Cambridge, UK) and a malondialdehyde (MDA) assay kit was purchased from Sigma Aldrich Co (St Louis, U.S.A). All rats of each group were provided with a standard diet and high-fat diet (Vrk Nutritionals Solutions, Pune, India) and had unrestricted access to water. The kits and reagents necessary for analyzing various biochemical parameters were sourced from Span Diagnostic Ltd., India.

4.2. Experimental Subject

4-8 weeks old male Wistar rats of 200-250g, each were housed individually in polypropylene cages maintained under standard laboratory conditions, i.e., temperature at 24 ± 2°C, relative humidity at 55 ± 5%, and a 12-hour light: dark cycle. The animal experiments were executed at the Animal Facility, Department of Pharmaceutical Technology, Jadavpur University, India (CPCSEA Reg. No.: 0367/01/C/CPCSEA, UGC, India). The animal experiment has been permitted by the Jadavpur University animal ethical committee (Proposal no.: JU/IAEC-22/36 dated 15.06.2023) and the principles of laboratory animal care were followed during the experiment.

4.3. Experimental design

A total of 24 male Wistar rats were randomly divided into four equal groups, with each group consisting of 6n animals. Group I served as the control group, receiving standard treatment without the induction of diabetes or administration of 4-phenylbutyric Acid (4-PBA). Group II was the diabetic control group, where diabetes was induced but no 4-PBA treatment was administered. Group 3 consisted of rats in which diabetes was induced, followed by treatment with a low dose of 4-PBA. Finally, Group 4 included rats with induced diabetes that were subsequently treated with a high dose of 4-PBA. The random allocation of rats ensured an equal number of subjects in each group, minimizing potential bias and allowing for a comprehensive evaluation of the effects of 4-PBA on diabetic nephropathy (Zhang et al., 2020; Liu et al., 2018). Diabetes will be induced in the diabetic and diabetic + 4-PBA treatment groups by a single injection of streptozotocin (STZ) (40 mg/kg) IP. The treatment group will receive oral administration of 4-PBA (50 mg/kg of body weight) for the low-dose treatment group and (100

mg/kg of body weight) for the high-dose treatment group for 2 weeks. Blood samples were collected from all rats of each group on the day of sacrifice for testing various biochemical parameters by the kits purchased from Span Diagnostic Ltd., India. At the end of the study, kidney tissue samples were collected for histological analysis to assess kidney damage and inflammation by using a Leica DFC 450 C microscope at a magnification of 40x (Dewanjee et al., 2013; Sahu et al., 2019). Data on blood glucose, kidney function, and biomarkers of inflammation and oxidative stress test were done by the kits purchased from HiMedia Laboratories, and data obtained were analyzed using statistical methods to determine the effects of 4-PBA on diabetic nephropathy. Histological analysis of kidney tissue samples was used to determine the effects of 4-PBA on kidney damage and inflammation. (Gupta and Gupta, 2020)

4.4. *In vivo* bioassay

4.4.1. Experimental setup

The *in vivo* study was performed according to established protocol in a lab (Das et al., 2010; Dua et al., 2016). A total of 24 male Wistar rats were grouped into four categories (n=6) and further procedures are as follows:

Group I: Control group, rats were fed with normal diet throughout the study.

Group II: Toxic group, rats were fed with the high-fat diet for 7 days following administration of STZ (35 mg/kg) on the 8th day, again fed a high-fat diet throughout the study.

Group III: Treatment group (low dose), rats were fed a high-fat diet for 7 days. On the 8th day, they received an administration of STZ (40 mg/kg). One week after the STZ administration, treatment with 4PBA at a high dose (50 mg/kg) began on the 15th day and continued for 2 weeks.

Group IV: Treatment group (high dose), rats were fed with a high-fat diet for 7 days following administration of STZ (40 mg/kg) on the 8th day, one week after administration STZ, 4PBA treatment with low dose (100 mg/kg) started from 15th day for 2 weeks.

The dose of 4 PBA was decided based on a comprehensive literature survey that examined the various therapeutic activities demonstrated by this postbiotic substance (Luo ZF et al., 2010). Food and water given to rats were monitored regularly. On the 29th day of the experiment, rats were sacrificed with prior fasting of 16 hrs. Rats were sacrificed under euthanasia using carbon dioxide (CO_2). Blood samples were collected from the retro-orbital sinus. The serum was meticulously obtained through a gentle centrifugation process, with a rotation speed of 10,000 rpm for 10 mins. The liver was isolated and cleansed by PBS. Each liver was sectioned in two

portions, one portion of liver tissue was preserved in 10% formalin for histological examinations, while the other portion was immediately homogenized in tris -HCL (0.01 M) with EDTA (0.001 M) buffer with PH = 7.4. The homogenized tissue was centrifuged at 12000 rpm for 30 min at 4oc, Supernatant was collected and used for the antioxidant assay.

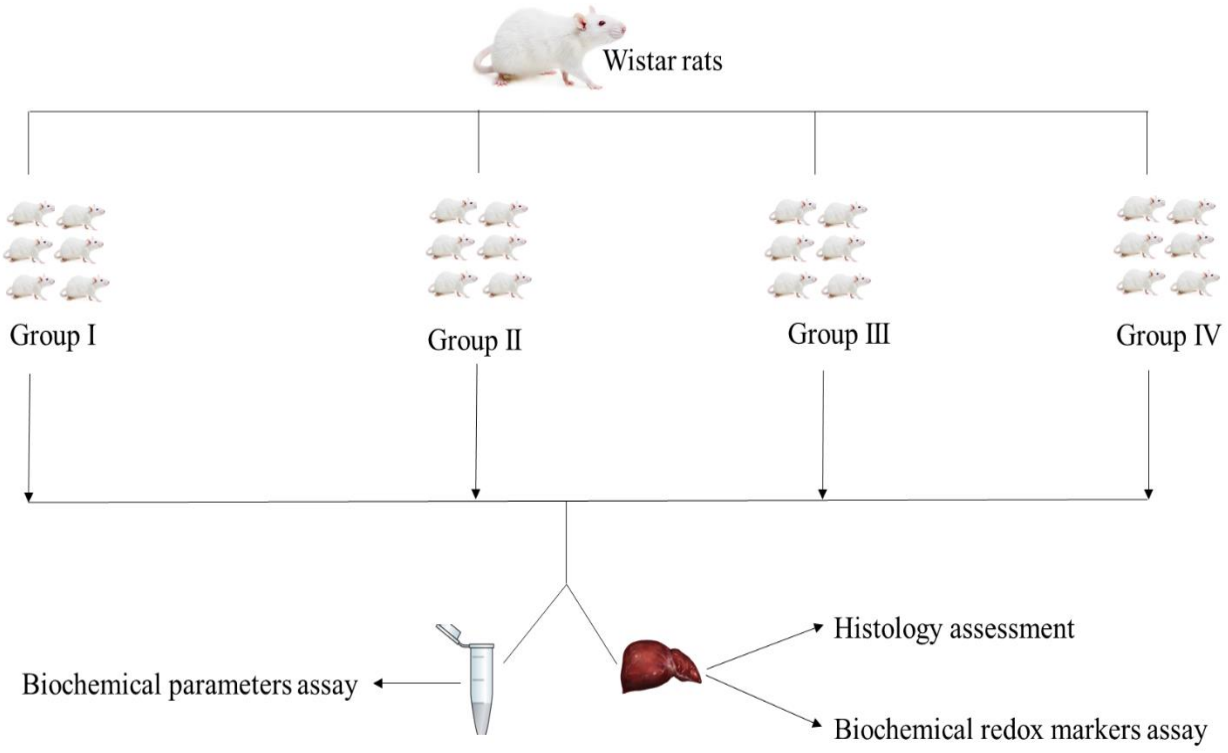

Figure 3. Schematic diagram of the experiment protocol

4.4.2. Estimation of biochemical parameters

The level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), Lipid profile, Blood Urea Nitrogen (BUN), Urea, Uric acid, Creatinine, C-reactive protein (Crp), Creatine kinase (CK), haemoglobin A1c (HBA1c) in the sera were determined using commercially available kits (Span Diagnostic Ltd., India) according to the instruction provided by the manufacturer.

4.4.3. Serum lipid profile evaluation

High-density lipids (HDL) and Triglyceride (TG) are tested and measured as per the manufacturer's instructions using commercially available enzymatic kits purchased from ARKRAY Healthcare Pvt Ltd, India. Density Lipid (LDL) level in blood serum is tested and calculated according to Friedewald's formula.

4.4.4. Assays of biochemical and Redox Markers in kidney

Glutathione (GSH), Superoxide dismutase (SOD), Malondialdehyde (MDA), and Total Protein in the liver of male Wistar rats in all the groups were measured as per the established protocols (Manna et al., 2022). Nitro blue tetrazolium (NBT) reduction per minute and H2O2 consumption per minute were expressed while measuring SOD as per the protocol of (Manna et al., 2022).

4.4.5. Histological studies

Histological analysis was conducted by taking a portion of liver tissue from all groups of experimental animals, tissues were thoroughly washed with ice-cold phosphate buffer of PH of 7.4, followed by fixing of tissue using 10% formalin solution and then mounted in paraffin blocks for sectioning. According to the established protocol the section was appropriately stained by haematoxylin and eosin (H&E) stains and images were taken using a Leica DFC 450 C microscope at a magnification of 40x (Dewanjee et al., 2013; Sahu et al., 2019).

4.4.6. Statistical Analysis

Experiments were performed in triplicate. The mean +- SD values were used to represent the data obtained from the experiment. The results underwent statistical analysis using one-way ANOVA followed by Dunnett's t-test with the assistance of GraphPad InStat (version 3.05), GraphPad Software, USA. Any p-value below 0.01- 0.05 was deemed significant.

Chapter 5

Results and Discussions

Contents

5. Results and Discussions

5.1 Results

5.1.1 Effect of 4-PBA on Diabetic nephropathy

5.1.1.1 Effect on body weight, Kidney weight

Before the administration of 4-PBA, all male Wistar rats across the experimental groups were weighed, establishing a baseline average body weight of 250g ± 40g. Following the induction of diabetes using Streptozotocin (STZ) at a dose of 40 mg/kg, diabetes was confirmed in all rats from Groups II, III, and IV within one week. This diabetes induction led to a slight but noticeable decrease in body weight across these diabetic groups, indicative of the metabolic disturbances and physiological stress associated with diabetes. In contrast, the body weight of rats in Group I, which were maintained on a normal diet without STZ treatment, remained relatively unchanged from the initial measurement, underscoring the stability of body weight under normal conditions. Subsequent to the induction of diabetes, treatment with 4-PBA was commenced in Groups III and IV. Rats in Group III were administered a low dose of 50 mg/kg of 4-PBA, while those in Group IV received a high dose of 100 mg/kg. The treatment lasted for two weeks, after which body weight measurements were taken again. Remarkably, the follow-up weights for both Groups III and IV showed a significant increase, with body weights approaching or even surpassing their initial pre-STZ levels. This recovery in body weight suggests that 4-PBA treatment has a beneficial effect on overall health and metabolic balance in diabetic rats, potentially reversing some of the weight loss associated with STZ-induced diabetes. During the sacrifice of the animals, kidney weights were also recorded. In the treatment groups (Groups III and IV), the kidney weights were found to be normal and comparable to those of the control group rats (Group I). This observation indicates that the administration of 4-PBA has a positive impact on kidney health, helping to restore kidney mass to near-normal levels after the damage inflicted by diabetes. Conversely, the kidney weights in the untreated diabetic rats from Group II remained significantly higher than those of the control group, reflecting persistent kidney damage and enlargement due to diabetic nephropathy. These results highlight the efficacy of 4-PBA treatment in ameliorating some of the adverse effects of diabetes. The increase in body weight and normalization of kidney weight in treated rats suggest that 4-PBA not only supports overall weight recovery but also plays a critical role in mitigating diabetes-induced renal damage, providing evidence for its potential therapeutic benefits in diabetic nephropathy.

5.1.1.2 Effect on blood parameters

Blood parameters are critical indicators of the body's pathological state, providing insights into various organ functions and metabolic processes. In the study, the effect of 4-PBA treatment on blood parameters in experimental rats was assessed and is illustrated in Figure 2. After administering Streptozotocin (STZ) at a dose of 40 mg/kg, there was a significant elevation in several key biochemical markers. The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) increased markedly. These enzymes are typically associated with liver function, and their elevated levels suggest potential liver damage or stress. Alongside these changes, the concentrations of blood urea nitrogen (BUN), urea, uric acid, and creatinine were also significantly higher. These markers are indicative of kidney function and their elevation points towards impaired renal performance, as these substances are normally filtered and excreted by the kidneys. Additionally, there was an increase in C-reactive protein (CRP), a known marker of inflammation. Elevated CRP levels reflect systemic inflammation, which is commonly observed in diabetic conditions and can contribute to further organ damage. Haemoglobin A1c (HbA1c) levels were also found to be significantly increased. HbA1c is a measure of long-term blood glucose levels and its elevation indicates chronic hyperglycaemia and poor glycaemic control. Following the administration of 4-PBA, a reduction in these elevated blood parameters was observed. Specifically, the levels of ALT and AST decreased, which may suggest an improvement in liver function or a reduction in liver damage. Similarly, the levels of BUN, urea, uric acid, and creatinine decreased, which points to a potential improvement in kidney function and a reduction in the accumulation of waste products. The decrease in CRP levels indicates a reduction in systemic inflammation, which is beneficial in managing diabetic complications. Furthermore, the reduction in HbA1c levels suggests an improvement in long-term blood glucose control, reflecting better management of hyperglycaemia. These observations indicate that the administration of 4-PBA positively affects various blood biochemical parameters, suggesting improvements in liver and kidney function, reduced inflammation, and better glycaemic control as shown in **Figure 4**.

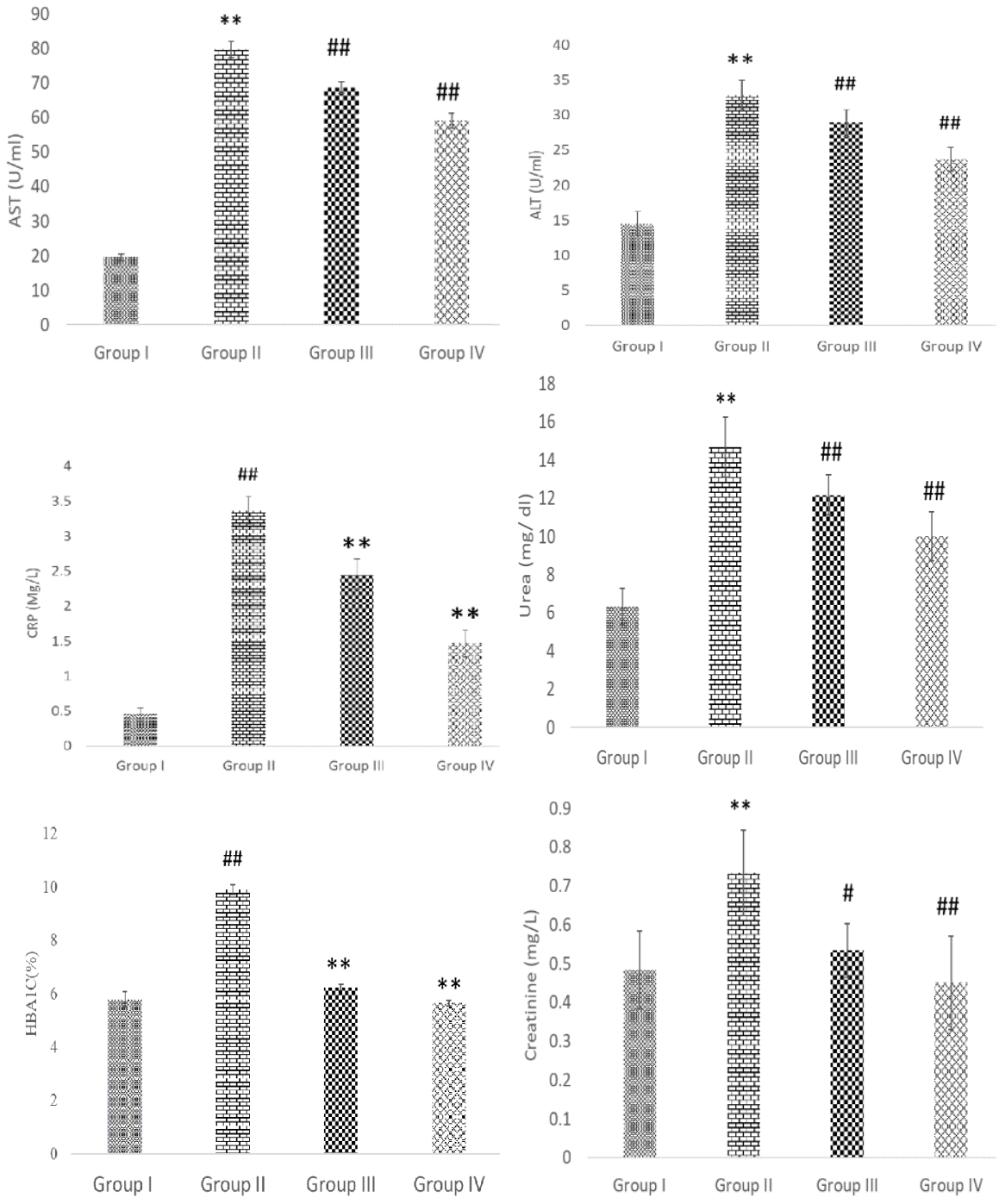

Figure 4. The effect of STZ and 4-PBA on blood parameters of control and experimental rats

5.1.1.3 Effects on serum lipid profile

Density Lipoprotein (HDL) and Triglyceride (TG) levels were measured using commercially available enzymatic kits from ARKRAY Healthcare Pvt Ltd, India, following the manufacturer's instructions. Low Density Lipoprotein (LDL) levels in the blood serum were calculated using Friedewald's formula. In the study, the analysis revealed that HDL and TG levels were elevated in the toxic group, which consisted of STZ-induced diabetic rats, compared to the control group. Similarly, LDL levels were also higher in the toxic group. In contrast, the treatment groups, that received 4-PBA, exhibited HDL, LDL, and TG levels that were closer to those observed in the control group, indicating a normalization of these lipid parameters. This suggests that the treatment with 4-PBA has a beneficial effect on lipid metabolism, bringing the levels of HDL, LDL, and TG closer to normal values observed in the non-diabetic control group **Figure 5.**

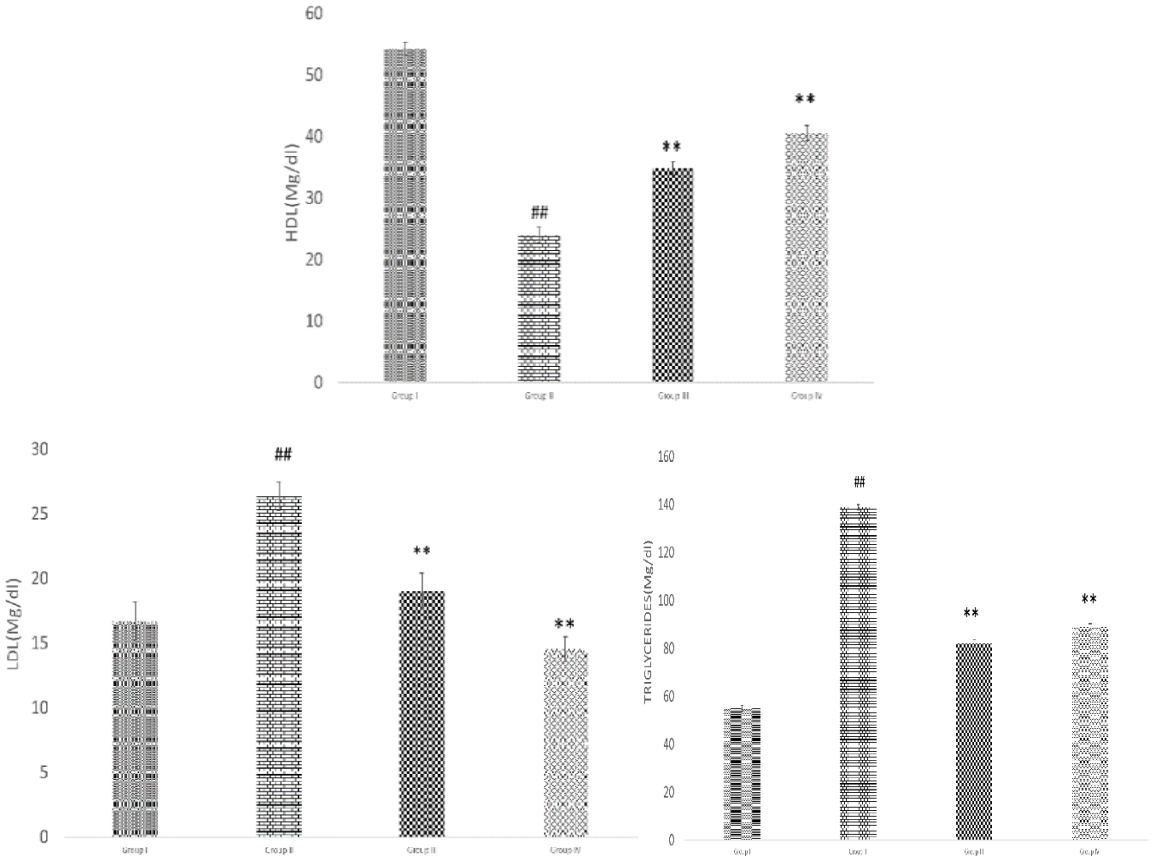

Figure 5. The effect of STZ and 4-PBA on serum HDL, LDL, triglycerides levels.

5.1.1.4 Effect on Oxidative Stress Parameters

The levels of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH), and total protein were measured according to established protocols (Manna et al., 2022). In the toxic group, which consisted of STZ-induced diabetic rats, these parameters were elevated compared to the control group. Specifically, SOD, an enzyme that helps mitigate oxidative stress, was increased, indicating a response to heightened oxidative damage. MDA, a marker of lipid peroxidation, was also elevated, reflecting increased oxidative stress. GSH, a key antioxidant, was found to be higher, which could be a compensatory response to oxidative damage. Total protein levels were also elevated, potentially due to increased production of stress-related proteins. In contrast, in the treatment group, which received 4-PBA, the levels of SOD, MDA, GSH, and total protein were found to be nearly similar to those in the control group. This suggests that 4-PBA treatment effectively modulates oxidative stress parameters, bringing them back to levels comparable to those observed in non-diabetic rats. This implies a reduction in oxidative stress and a potential restoration of normal antioxidant defenses and protein levels in the treatment group as shown in **Figure 6.**

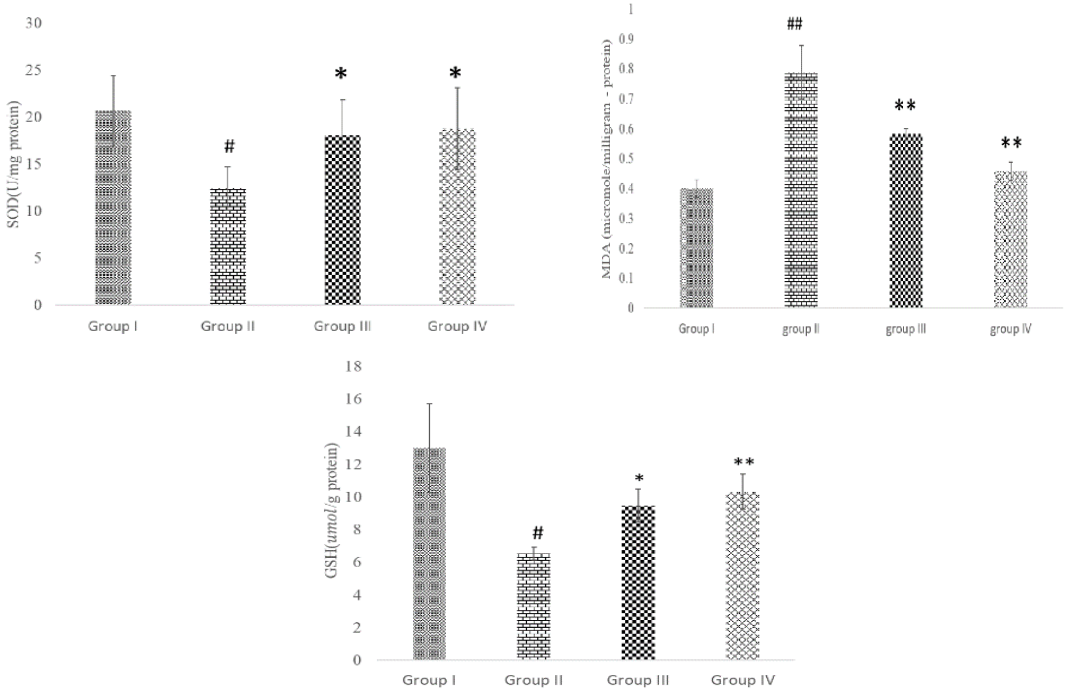

Figure 6. The effect on GSH, SOD, and MDA in the kidney of control and experimental rats

5.1.1.5 Effect on histological parameters:

Histopathological Findings:

Kidneys from control rats (Group I) display a normal histological structure, characterized by intact glomeruli with well-defined capillary loops and a normal mesangial matrix. The renal tubules are regular, showing no signs of degeneration or dilation, and the interstitial space remains minimal without any inflammatory cell infiltration. This indicates a healthy kidney with no apparent pathology. Conversely, in STZ-induced diabetic rats (Group II), there are pronounced histopathological changes in the kidney tissues. The glomeruli are notably enlarged, with a marked increase in mesangial matrix expansion and thickening of the glomerular basement membrane. These alterations, along with occasional glomerulosclerosis, are indicative of diabetic nephropathy, a common complication associated with diabetes that can lead to progressive kidney damage and dysfunction. In the groups of STZ-induced diabetic rats treated with 4-PBA Groups III and IV, low dose (50mg/kg) and high dose (100mg/kg) respectively, there is evidence of partial mitigation of the kidney damage with respect to dose proportion induced by diabetes. The treatment leads to a noticeable reduction in mesangial matrix expansion and glomerular basement membrane thickening. Additionally, glomerulosclerosis is observed less frequently compared to the untreated diabetic group. This suggests that 4-PBA has a protective or therapeutic effect on diabetic nephropathy, potentially improving the histological appearance of the kidneys and alleviating some of the pathological changes associated as shown in **Figure 7**.

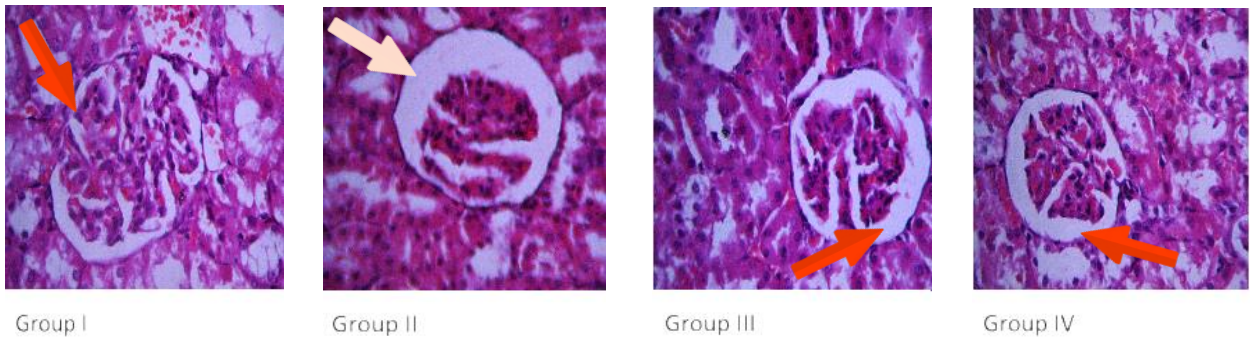

Figure 7. Haematoxylin- eosin (H & E) histo-pathological observation of kidney in rats. The kidney sections of normal rats (Group I) showed intact glomerulus While the Toxic rats (Group II) showed enlarged glomerulus. The treatment groups (Group III and Group IV) showed a slight improvement in the status of the glomerulus.

5.2. Discussion

The present study offers an in-depth evaluation of the therapeutic potential of 4-PBA in mitigating the effects of diabetic nephropathy, a serious and progressive complication of diabetes mellitus. The investigation focused on understanding how 4-PBA affects a range of physiological and biochemical parameters, including body weight, kidney weight, blood biochemical markers, serum lipid profiles, oxidative stress markers, and the histological structure of kidney tissues in diabetic rats. The findings not only contribute to the existing body of knowledge but also open new avenues for the potential application of 4-PBA in treating diabetes-related complications.The induction of diabetes in Wistar rats through STZ is well-documented to cause significant metabolic disturbances, leading to weight loss. This weight loss is primarily attributed to insulin deficiency, which results in increased proteolysis and lipolysis, thereby causing muscle wasting and fat loss. In our study, rats subjected to STZ-induced diabetes exhibited a noticeable reduction in body weight, aligning with previous studies that reported similar outcomes. For instance, a study documented significant weight loss in diabetic rats, which was primarily due to uncontrolled catabolism of proteins and fats (Arulselvan et al.,2012).Interestingly, when 4-PBA was administered to these diabetic rats, a remarkable recovery in body weight was observed. The rats treated with both low (50 mg/kg) and high (100 mg/kg) doses of 4-PBA regained their lost body weight, nearly reaching or even surpassing their initial pre-diabetic levels. This weight recovery suggests that 4-PBA may play a role in restoring metabolic homeostasis, possibly by enhancing insulin sensitivity or reducing the metabolic stress associated with diabetes. The ability of 4-PBA to mitigate weight loss in diabetic conditions could also be linked to its known function as a chemical chaperone, which reduces ER stress, a condition often exacerbated in diabetes.In addition to body weight, kidney weight serves as a critical marker of diabetic nephropathy, with diabetes often leading to renal hypertrophy due to glomerular and tubular hypertrophy as well as increased extracellular matrix deposition. In this study, untreated diabetic rats showed significantly increased kidney weights, which is consistent with the literature that highlights kidney enlargement as a hallmark of diabetic nephropathy (Tervaert et al., 2010). The administration of 4-PBA, however, normalized the kidney weights in the treated groups, bringing them closer to the levels observed in the non-diabetic control group. This finding is particularly important as it suggests that 4-PBA not only protects against diabetes-induced kidney damage but may also reverse some of the hypertrophic changes

associated with diabetic nephropathy. Similar outcomes were observed in studies by Ozcan et al. (2006), where chemical chaperones like 4-PBA were found to mitigate ER stress and, consequently, reduce kidney hypertrophy in diabetic models (Ozcan et al., 2004).The analysis of blood biochemical markers provides crucial insights into the functional status of various organs, particularly the liver, and kidneys, which are often adversely affected in diabetes. In this study, the STZ-induced diabetic rats exhibited significant elevations in several key biochemical markers, including ALT, AST, BUN, urea, uric acid, and creatinine. Elevated levels of ALT and AST are indicative of liver stress or damage, which is a common consequence of chronic hyperglycemia in diabetes. High levels of BUN, urea, uric acid, and creatinine, on the other hand, reflect impaired kidney function, as these are waste products normally filtered and excreted by the kidneys. The increase in these markers in diabetic rats aligns with previous studies that have reported similar biochemical changes in diabetic conditions, highlighting the detrimental effects of uncontrolled diabetes on both liver and kidney function.The administration of 4-PBA resulted in a significant reduction in these elevated biochemical markers, suggesting an improvement in both liver and kidney function. The decrease in ALT and AST levels points to a potential alleviation of liver stress, while the reduction in BUN, urea, uric acid, and creatinine levels indicates improved kidney function. This is particularly noteworthy as it suggests that 4-PBA may help in mitigating the organ damage typically seen in diabetes. These findings are in line with the study by Zhang and Kaufman (2008), which demonstrated that 4-PBA could reduce ER stress, thereby improving the function of vital organs like the liver and kidneys in diabetic conditions (Zhang and Kaufman,2008).Additionally, the study observed elevated levels of CRP and HbA1c in diabetic rats, both of which are critical markers in the context of diabetes. CRP is a well-known marker of systemic inflammation, which is often elevated in diabetic conditions and contributes to the progression of complications like nephropathy. Similarly, HbA1c serves as a reliable marker of long-term blood glucose levels, with higher levels indicating poor glycemic control and chronic hyperglycemia. The reduction in CRP and HbA1c levels following 4-PBA treatment is particularly significant, as it suggests that 4-PBA not only improves glycemic control but also reduces systemic inflammation, which is crucial for preventing or slowing the progression of diabetic complications (King, 2008).Dyslipidemia, characterized by elevated levels of LDL, TG, and reduced levels of HDL, is a common feature of diabetes and plays a pivotal role in the development of cardiovascular complications. In this study, the STZ-induced

diabetic rats exhibited significant dyslipidemia, with elevated levels of LDL and TG, and altered HDL levels. These lipid abnormalities are well-documented in the literature, where hyperglycemia-induced alterations in lipid metabolism contribute to the progression of atherosclerosis and other vascular complications (Mooradian, 2009).The administration of 4-PBA led to a normalization of the lipid profile in treated rats, bringing LDL, TG, and HDL levels closer to those observed in non-diabetic control rats. This finding suggests that 4-PBA has a beneficial effect on lipid metabolism, possibly by enhancing the activity of lipid-regulating enzymes or by reducing oxidative stress, which is known to exacerbate dyslipidemia in diabetic conditions. The improvement in lipid profiles is crucial, as it indicates a potential reduction in the cardiovascular risks associated with diabetes, which is supported by studies such as those by Erion and Shulman (2010) that highlight the role of 4-PBA in modulating lipid metabolism and reducing cardiovascular risks in metabolic diseases.Oxidative stress is a key pathogenic factor in diabetic nephropathy, where an imbalance between the production of ROS and the antioxidant defense system leads to cellular damage and tissue injury. In this study, diabetic rats exhibited elevated levels of oxidative stress markers, including SOD, MDA, and GSH, reflecting a heightened oxidative state. The increase in SOD, an antioxidant enzyme, suggests a compensatory response to oxidative stress, while elevated MDA levels indicate increased lipid peroxidation, a damaging process that affects cellular membranes. The high levels of GSH, a critical antioxidant, may also reflect an adaptive response to counteract the oxidative damage.The treatment with 4-PBA resulted in a significant reduction in these oxidative stress markers, bringing them closer to the levels observed in non-diabetic control rats. This suggests that 4-PBA effectively modulates the oxidative stress response, likely through its role in reducing ER stress and enhancing the antioxidant defense system. The reduction in oxidative stress is particularly important in the context of diabetic nephropathy, as it implies a potential slowing of disease progression and preservation of renal function. These findings align with the work (Ozcan et al.,2012), which demonstrated the antioxidant properties of 4-PBA and its ability to mitigate oxidative damage in diabetic models (Ozcan et al., 2012). Histological examination of kidney tissues provides a direct assessment of the structural changes associated with diabetic nephropathy. In this study, untreated diabetic rats exhibited significant histopathological alterations, including glomerular enlargement, mesangial matrix expansion, and thickening of the glomerular basement membrane. These changes are characteristic of diabetic nephropathy and

are indicative of progressive kidney damage, which can eventually lead to renal failure if left unchecked. The presence of glomerulosclerosis, or scarring within the glomeruli, further underscores the severity of the nephropathic changes observed in diabetic rats.The administration of 4-PBA to diabetic rats resulted in a noticeable improvement in kidney histology, with a reduction in glomerular enlargement, mesangial expansion, and basement membrane thickening. Additionally, the frequency and severity of glomerulosclerosis were reduced in the 4-PBA-treated groups, suggesting that 4-PBA has a protective effect on the kidneys. These histological improvements are significant as they indicate that 4-PBA may help in preserving kidney structure and function in the context of diabetes, potentially delaying the onset of end-stage renal disease. The ability of 4-PBA to ameliorate structural damage in the kidneys is consistent with findings from studies by Hotamisligil, which demonstrated the role of 4-PBA in reducing fibrosis and inflammation in various tissues by modulating the unfolded protein response (Hotamisligil, 2010).

5.3. Comparative Analysis with Existing Therapies

5.3.1. Comparison with RAAS Inhibitors and SGLT2 Inhibitors

Current therapies for DN, including RAAS inhibitors and SGLT2 inhibitors, primarily target hemodynamic and metabolic pathways (Lewis et al., 1993). While these therapies are effective in slowing DN progression, they do not address the underlying cellular stress responses that drive the disease. 4-PBA, by targeting ER stress, inflammation, and oxidative stress, offers a complementary approach that could enhance the efficacy of existing treatments. Combining 4-PBA with standard therapies may provide a more comprehensive strategy to manage DN, potentially leading to better outcomes.

5.3.2. Potential Synergies and Combination Therapy

Given the different mechanisms of action, there is potential for synergy between 4-PBA and existing DN therapies. For example, combining 4-PBA with an SGLT2 inhibitor might provide both metabolic and cellular protection, reducing the progression of DN more effectively than either agent alone. This hypothesis is supported by studies that have shown the benefits of combining different therapeutic agents to target multiple pathways in DN (Roscioni et al., 2014). Future research should explore the efficacy and safety of such combination therapies in clinical settings.

5.4. Implications for Clinical Practice

5.4.1. Translational Potential and Challenges

The promising results from this study suggest that 4-PBA could be a valuable addition to the therapeutic options for DN. However, translating these findings into clinical practice presents several challenges. Determining the appropriate dosage and treatment duration for humans is critical, as the doses used in animal studies may not directly translate to human applications. Additionally, the long-term safety of 4-PBA needs to be evaluated, particularly its effects on liver function and other metabolic processes, as chronic administration may pose risks.

5.4.2. Personalized Medicine and Biomarker Development

Given the heterogeneity of DN in humans, personalized approaches may be necessary to optimize treatment outcomes. Identifying biomarkers that predict response to 4-PBA could enable clinicians to tailor therapy to individual patients, maximizing benefits while minimizing risks. This approach is increasingly recognized as crucial in managing complex, multifactorial diseases like DN, where patient response to treatment can vary widely.

5.5 Future Directions for Research

5.5.1. Long-term Studies and Clinical Trials

Future research should focus on evaluating the long-term effects of 4-PBA treatment in DN, including its impact on renal survival, overall mortality, and quality of life. Long-term studies in animal models, as well as well-designed clinical trials, are necessary to establish the safety and efficacy of 4-PBA in human patients with DN. These trials should include diverse populations to ensure generalizability and explore potential combination therapies with other nephroprotective agents.

5.5.2. Exploring Mechanistic Pathways

Further mechanistic studies are needed to fully understand how 4-PBA modulates ER stress, inflammation, and oxidative stress in DN. Understanding these mechanisms could lead to the development of more targeted therapies and identify additional pathways that could be modulated for therapeutic benefit. Additionally, exploring the interactions between 4-PBA and other cellular pathways involved in DN could provide a more comprehensive understanding of its therapeutic potential.

Chapter 6

Conclusion

6.Conclusion

The study reveals that 4-PBA holds significant therapeutic potential in treating diabetic nephropathy by improving various physiological and biochemical parameters, such as body and kidney weight, blood markers, lipid profiles, oxidative stress levels, and kidney histology in diabetic rats. 4-PBA effectively counteracts the metabolic disturbances of diabetes, restoring metabolic balance and protecting against organ damage by reducing liver and kidney stress markers. Additionally, 4-PBA lowers systemic inflammation and oxidative stress, key contributors to DN, and shows potential in preserving kidney structure by mitigating histopathological changes. Its unique mechanism, targeting ER stress, inflammation, and oxidative stress, complements existing DN therapies like RAAS and SGLT2 inhibitors, suggesting that 4-PBA could enhance treatment outcomes when used in combination. However, further research, including long-term studies and clinical trials, is needed to confirm its safety and efficacy in humans and to explore its potential in personalized medicine approaches.

Chapter 7

References

7. References

Abdali D, Samson SE, Grover AK. How effective are antioxidant supplements in obesity and diabetes? Med Princ Pract. 2015;24(3):201-15. doi: 10.1159/000375305.

Alam S, Hasan MK, Neaz S, Hussain N, Hossain MF, Rahman T. Diabetes Mellitus: insights from epidemiology, biochemistry, risk factors, diagnosis, complications and comprehensive management. Diabetology. 2021 Apr 16;2(2):36-50.

Aloke C, Egwu CO, Aja PM, Obasi NA, Chukwu J, Akumadu BO, Ogbu PN, Achilonu I. Current Advances in the Management of Diabetes Mellitus. Biomedicines. 2022 Sep 29;10(10):2436. doi: 10.3390/biomedicines10102436.

Alwan A, Maclean DR, Riley LM, d'Espaignet ET, Mathers CD, Stevens GA, Bettcher D. Monitoring and surveillance of chronic non-communicable diseases: progress and capacity in high-burden countries. Lancet. 2010 Nov 27;376(9755):1861-8. doi: 10.1016/S0140-6736(10)61853-3.

American Diabetes Association. Classification and diagnosis of diabetes. Diabetes Care. 2017;40(Suppl 1):S11–S24. doi:10.2337/dc17-S005

Amores-Arrocha A, Roldán A, Jiménez-Cantizano A, Caro I, Palacios V. Evaluation of the use of multiflora bee pollen on the volatile compounds and sensorial profile of Palomino fino and Riesling white young wines. Food Res Int. 2018 Mar;105:197-209. doi: 10.1016/j.foodres.2017.11.013.

Antonetti, D.A.; Klein, R.; Gardner, T.W. Diabetic retinopathy. N. Engl. J. Med. 2012, 366, 1227–1239.

Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress-A concise review. Saudi Pharm J. 2016 Sep;24(5):547-553. doi: 10.1016/j.jsps.2015.03.013.

Assaggaf MA, Kantarci A, Sume SS, Trackman PC. Prevention of phenytoin-induced gingival overgrowth by lovastatin in mice. Am J Pathol. 2015 Jun;185(6):1588-99. doi: 10.1016/j.ajpath.2015.02.004.

Barbagallo M, Dominguez LJ. Magnesium and type 2 diabetes. World J Diabetes. 2015 Aug 25;6(10):1152-7. doi: 10.4239/wjd.v6.i10.1152.

Barros CP, Pires RPS, Guimarães JT, Abud YKD, Almada CN, Pimentel TC, Sant'Anna C, De-Melo LDB, Duarte MCKH, Silva MC, Sant'Ana AS, Freitas MQ, Cruz AG. Ohmic heating as a method of obtaining paraprobiotics: Impacts on cell structure and viability by

flow cytometry. Food Res Int. 2021 Feb;140:110061. doi: 10.1016/j.foodres.2020.110061.

Cabello-Olmo M, Araña M, Urtasun R, Encio IJ, Barajas M. Role of Postbiotics in Diabetes Mellitus: Current Knowledge and Future Perspectives. Foods. 2021 Jul 8;10(7):1590. doi: 10.3390/foods10071590.

Cao Y, Liu Z, Xiao W, Gu Z, Xiao G, Yuan F, Chen F, Pei Y, Li H, Su L. 4-Phenylbutyrate Prevents Endoplasmic Reticulum Stress-Mediated Apoptosis Induced by Heatstroke in the Intestines of Mice. Shock. 2020 Jul;54(1):102-109. doi: 10.1097/SHK.0000000000001419.

Chaudhury A, Duvoor C, Reddy Dendi VS, Kraleti S, Chada A, Ravilla R, Marco A, Shekhawat NS, Montales MT, Kuriakose K, Sasapu A, Beebe A, Patil N, Musham CK, Lohani GP, Mirza W. Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management. Front Endocrinol (Lausanne). 2017 Jan 24;8:6. doi: 10.3389/fendo.2017.00006.

Chen CH, Shih PC, Lin HY, Wang PK, Pan PT, Chuang CW, Kao MC. 4-Phenylbutyric acid protects against vasculitic peripheral neuropathy induced by ischaemia-reperfusion through attenuating endoplasmic reticulum stress. Inflammopharmacology. 2019 Aug;27(4):713-722. doi: 10.1007/s10787-019-00604-6.

Chen J, Yang F, Yu X, Yu Y, Gong Y. Cyclosporine A promotes cell proliferation, collagen and α-smooth muscle actin expressions in rat gingival fibroblasts by Smad3 activation and miR-29b suppression. J Periodontal Res. 2016 Dec;51(6):735-747. doi: 10.1111/jre.12350.

Chiang CK, Hsu SP, Wu CT, Huang JW, Cheng HT, Chang YW, Hung KY, Wu KD, Liu SH. Endoplasmic reticulum stress implicated in the development of renal fibrosis. Mol Med. 2011;17(11-12):1295-305. doi: 10.2119/molmed.2011.00131.

Cuevas-González PF, Liceaga AM, Aguilar-Toalá JE. Postbiotics and paraprobiotics: From concepts to applications. Food Res Int. 2020 Oct;136:109502. doi: 10.1016/j.foodres.2020.109502.

Das Gupta K, Shakespear MR, Iyer A, Fairlie DP, Sweet MJ. Histone deacetylases in monocyte/macrophage development, activation and metabolism: refining HDAC targets

for inflammatory and infectious diseases. Clin Transl Immunology. 2016 Jan 29;5(1):e62. doi: 10.1038/cti.2015.46.

Domon H, Takahashi N, Honda T, Nakajima T, Tabeta K, Abiko Y, Yamazaki K. Up-regulation of the endoplasmic reticulum stress-response in periodontal disease. Clin Chim Acta. 2009 Mar;401(1-2):134-40. doi: 10.1016/j.cca.2008.12.007.

Dromparis P, Paulin R, Stenson TH, Haromy A, Sutendra G, Michelakis ED. Attenuating endoplasmic reticulum stress as a novel therapeutic strategy in pulmonary hypertension. Circulation. 2013 Jan 1;127(1):115-25. doi: 10.1161/CIRCULATIONAHA.112.133413.

Falk E, Nakano M, Bentzon JF, Finn AV, Virmani R. Update on acute coronary syndromes: the pathologists' view. Eur Heart J. 2013 Mar;34(10):719-28. doi: 10.1093/eurheartj/ehs411.

Fan Y, Lee K, Wang N, He JC. The Role of Endoplasmic Reticulum Stress in Diabetic Nephropathy. Curr Diab Rep. 2017 Mar;17(3):17. doi: 10.1007/s11892-017-0842-y.

Fijalkowska I, Xu W, Comhair SA, Janocha AJ, Mavrakis LA, Krishnamachary B, Zhen L, Mao T, Richter A, Erzurum SC, Tuder RM. Hypoxia inducible-factor1alpha regulates the metabolic shift of pulmonary hypertensive endothelial cells. Am J Pathol. 2010 Mar;176(3):1130-8. doi: 10.2353/ajpath.2010.090832.

Fong W, Li Q, Yu J. Gut microbiota modulation: a novel strategy for prevention and treatment of colorectal cancer. Oncogene. 2020 Jun;39(26):4925-4943. doi: 10.1038/s41388-020-1341-1.

Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev. 2013 Jan;93(1):137-88. doi: 10.1152/physrev.00045.2011.

Frump AL, Lowery JW, Hamid R, Austin ED, de Caestecker M. Abnormal trafficking of endogenously expressed BMPR2 mutant allelic products in patients with heritable pulmonary arterial hypertension. PLoS One. 2013 Nov 5;8(11):e80319. doi: 10.1371/journal.pone.0080319.

Galib R, Dang P, Kumar V, Rana R, Yadav P, Prajapati PK. Patterns of concomitant use of *Ayurveda* and conventional anti-diabetic formulations - Experiences at a tertiary care *Ayurveda* hospital, India. Ayu. 2020 Apr-Jun;41(2):72-78. doi: 10.4103/ayu.AYU_81_20.

Gardner BM, Pincus D, Gotthardt K, Gallagher CM, Walter P. Endoplasmic reticulum stress sensing in the unfolded protein response. Cold Spring Harb Perspect Biol. 2013 Mar 1;5(3):a013169. doi: 10.1101/cshperspect.a013169.

Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010 Oct 29;107(9):1058-70. doi: 10.1161/CIRCRESAHA.110.223545.

Gilbert RE, Cooper ME. The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of glomerular injury? Kidney Int. 1999 Nov;56(5):1627-37. doi: 10.1046/j.1523-1755.1999.00721.x.

Gouws C, Hamman JH. What are the dangers of drug interactions with herbal medicines? Expert Opin Drug Metab Toxicol. 2020 Mar;16(3):165-167. doi: 10.1080/17425255.2020.1733969.

Gurunathan S, Thangaraj P, Kim JH. Postbiotics: Functional Food Materials and Therapeutic Agents for Cancer, Diabetes, and Inflammatory Diseases. Foods. 2023 Dec 26;13(1):89. doi: 10.3390/foods13010089

Gurunathan S, Thangaraj P, Kim JH. Postbiotics: Functional Food Materials and Therapeutic Agents for Cancer, Diabetes, and Inflammatory Diseases. Foods. 2023 Dec 26;13(1):89. doi: 10.3390/foods13010089.

Hirota K, Semenza GL. Regulation of angiogenesis by hypoxia-inducible factor 1. Crit Rev Oncol Hematol. 2006 Jul;59(1):15-26. doi: 10.1016/j.critrevonc.2005.12.003.

Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell. 2010 Mar 19;140(6):900-17. doi: 10.1016/j.cell.2010.02.034.

Hurrle S, Hsu WH. The etiology of oxidative stress in insulin resistance. Biomed J. 2017 Oct;40(5):257-262. doi: 10.1016/j.bj.2017.06.007.

Inagi R. Endoplasmic reticulum stress as a progression factor for kidney injury. Curr Opin Pharmacol. 2010 Apr;10(2):156-65. doi: 10.1016/j.coph.2009.11.006. Epub 2010 Jan 4.

Inceoglu B, Bettaieb A, Trindade da Silva CA, Lee KS, Haj FG, Hammock BD. Endoplasmic reticulum stress in the peripheral nervous system is a significant driver of neuropathic pain. Proc Natl Acad Sci U S A. 2015 Jul 21;112(29):9082-7. doi: 10.1073/pnas.1510137112.

Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Arch Toxicol. 2023 Oct;97(10):2499-2574. doi: 10.1007/s00204-023-03562-9.

Khan MM, Yang WL, Wang P. ENDOPLASMIC RETICULUM STRESS IN SEPSIS. Shock. 2015 Oct;44(4):294-304. doi: 10.1097/SHK.0000000000000425.

Khan SR. Role of renal epithelial cells in the initiation of calcium oxalate stones. Nephron Exp Nephrol. 2004;98(2):e55-60. doi: 10.1159/000080257.

Kolb PS, Ayaub EA, Zhou W, Yum V, Dickhout JG, Ask K. The therapeutic effects of 4-phenylbutyric acid in maintaining proteostasis. Int J Biochem Cell Biol. 2015 Apr;61:45-52. doi: 10.1016/j.biocel.2015.01.015.

Kolb PS, Ayaub EA, Zhou W, Yum V, Dickhout JG, Ask K. The therapeutic effects of 4-phenylbutyric acid in maintaining proteostasis. Int J Biochem Cell Biol. 2015 Apr;61:45-52. doi: 10.1016/j.biocel.2015.01.015.

Kudo T, Kanemoto S, Hara H, Morimoto N, Morihara T, Kimura R, Tabira T, Imaizumi K, Takeda M. A molecular chaperone inducer protects neurons from ER stress. Cell Death Differ. 2008 Feb;15(2):364-75. doi: 10.1038/sj.cdd.4402276.

Kullisaar T, Zilmer M, Mikelsaar M, Vihalemm T, Annuk H, Kairane C, Kilk A. Two antioxidative lactobacilli strains as promising probiotics. Int J Food Microbiol. 2002 Feb 5;72(3):215-24. doi: 10.1016/s0168-1605(01)00674-2.

Kusaczuk M, Bartoszewicz M, Cechowska-Pasko M. Phenylbutyric Acid: simple structure - multiple effects. Curr Pharm Des. 2015;21(16):2147-66. doi: 10.2174/1381612821666150105160059.

Kwak KH, Han CG, Lee SH, Jeon Y, Park SS, Kim SO, Baek WY, Hong JG, Lim DG. Reactive oxygen species in rats with chronic post-ischemia pain. Acta Anaesthesiol Scand. 2009 May;53(5):648-56. doi: 10.1111/j.1399-6576.2009.01937.x.

Laybutt DR, Preston AM, Akerfeldt MC, Kench JG, Busch AK, Biankin AV, Biden TJ. Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. Diabetologia. 2007 Apr;50(4):752-63. doi: 10.1007/s00125-006-0590-z.

Leslie RD, Williams R, Pozzilli P. Clinical review: Type 1 diabetes and latent autoimmune diabetes in adults: one end of the rainbow. J Clin Endocrinol Metab. 2006 May;91(5):1654-9. doi: 10.1210/jc.2005-1623.

Lévy-Marchal C, Patterson CC, Green A; EURODIAB ACE Study Group. Europe and Diabetes. Geographical variation of presentation at diagnosis of type I diabetes in children: the EURODIAB study. European and Dibetes. Diabetologia. 2001 Oct;44 Suppl 3:B75-80. doi: 10.1007/pl00002958.

Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. N Engl J Med. 1993 Nov 11;329(20):1456-62. doi: 10.1056/NEJM199311113292004. Erratum in: N Engl J Med 1993 Jan 13;330(2):152.

Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I; Collaborative Study Group. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. N Engl J Med. 2001 Sep 20;345(12):851-60. doi: 10.1056/NEJMoa011303

Li S, Zhao F, Cheng S, Wang X, Hao Y. Uric acid-induced endoplasmic reticulum stress triggers phenotypic change in rat glomerular mesangial cells. Nephrology (Carlton). 2013 Oct;18(10):682-9. doi: 10.1111/nep.12127.

Lim, A.K. Diabetic nephropathy—Complications and treatment. Int. J. Nephrol. Renov. Dis. 2014, 7, 361–381.

Liu L, Wu H, Zang J, Yang G, Zhu Y, Wu Y, Chen X, Lan D, Li T. 4-Phenylbutyric Acid Reveals Good Beneficial Effects on Vital Organ Function via Anti-Endoplasmic Reticulum Stress in Septic Rats. Crit Care Med. 2016 Aug;44(8):e689-701. doi: 10.1097/CCM.0000000000001662.

Lovic D, Piperidou A, Zografou I, Grassos H, Pittaras A, Manolis A. The Growing Epidemic of Diabetes Mellitus. Curr Vasc Pharmacol. 2020;18(2):104-109. doi: 10.2174/1570161117666190405165911.

Lynn EG, Lhoták Š, Lebeau P, Byun JH, Chen J, Platko K, Shi C, O'Brien ER, Austin RC. 4-Phenylbutyrate protects against atherosclerotic lesion growth by increasing the expression of HSP25 in macrophages and in the circulation of *Apoe-/-* mice. FASEB J. 2019 Jul;33(7):8406-8422. doi: 10.1096/fj.201802293RR.

Ma YY, Di ZM, Cao Q, Xu WS, Bi SX, Yu JS, Shen YJ, Yu YQ, Shen YX, Feng LJ. Xanthatin induces glioma cell apoptosis and inhibits tumor growth via activating endoplasmic

reticulum stress-dependent CHOP pathway. Acta Pharmacol Sin. 2020 Mar;41(3):404-414. doi: 10.1038/s41401-019-0318-5.

Marciniak SJ, Chambers JE, Ron D. Pharmacological targeting of endoplasmic reticulum stress in disease. Nat Rev Drug Discov. 2022 Feb;21(2):115-140. doi: 10.1038/s41573-021-00320-3.

Mimori S, Okuma Y, Kaneko M, Kawada K, Hosoi T, Ozawa K, Nomura Y, Hamana H. Protective effects of 4-phenylbutyrate derivatives on the neuronal cell death and endoplasmic reticulum stress. Biol Pharm Bull. 2012;35(1):84-90. doi: 10.1248/bpb.35.84.

Morran MP, Vonberg A, Khadra A, Pietropaolo M. Immunogenetics of type 1 diabetes mellitus. Mol Aspects Med. 2015 Apr;42:42-60. doi: 10.1016/j.mam.2014.12.004.

Nakagawa T, Mazzali M, Kang DH, Kanellis J, Watanabe S, Sanchez-Lozada LG, Rodriguez-Iturbe B, Herrera-Acosta J, Johnson RJ. Hyperuricemia causes glomerular hypertrophy in the rat. Am J Nephrol. 2003 Jan-Feb;23(1):2-7. doi: 10.1159/000066303.

Nataraj BH, Ali SA, Behare PV, Yadav H. Postbiotics-parabiotics: the new horizons in microbial biotherapy and functional foods. Microb Cell Fact. 2020 Aug 20;19(1):168. doi: 10.1186/s12934-020-01426-w.

Navarro-González JF, Mora-Fernández C, Muros de Fuentes M, García-Pérez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. Nat Rev Nephrol. 2011 Jun;7(6):327-40. doi: 10.1038/nrneph.2011.51.

Ni L, Yuan C, Wu X. Endoplasmic Reticulum Stress in Diabetic Nephrology: Regulation, Pathological Role, and Therapeutic Potential. Oxid Med Cell Longev. 2021 Aug 2;2021:7277966. doi: 10.1155/2021/7277966.

O'Connell S, Tuite N, Slattery C, Ryan MP, McMorrow T. Cyclosporine A--induced oxidative stress in human renal mesangial cells: a role for ERK 1/2 MAPK signaling. Toxicol Sci. 2012 Mar;126(1):101-13. doi: 10.1093/toxsci/kfr330.

Ohanson NJ, Pretorius D. Compliance of medical practitioners with diabetic treatment guidelines in West Rand, Gauteng. South African Family Practice. 2023 Apr 23;65(2).

Omura T, Kaneko M, Okuma Y, Matsubara K, Nomura Y. Endoplasmic reticulum stress and Parkinson's disease: the role of HRD1 in averting apoptosis in neurodegenerative disease. Oxid Med Cell Longev. 2013;2013:239854. doi: 10.1155/2013/239854.

O'Neal KS, Johnson JL, Panak RL. Recognizing and Appropriately Treating Latent Autoimmune Diabetes in Adults. Diabetes Spectr. 2016 Nov;29(4):249-252. doi: 10.2337/ds15-0047.

Onyiriuka, A.N.; Ifebi, E. Ketoacidosis at diagnosis of type 1 diabetes in children and adolescents: Frequency and clinical characteristics. J. Diabetes Metab. Disord. 2013, 12, 47

Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Görgün CZ, Hotamisligil GS. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science. 2006 Aug 25;313(5790):1137-40. doi: 10.1126/science.1128294.

Parbin S, Shilpi A, Kar S, Pradhan N, Sengupta D, Deb M, Rath SK, Patra SK. Insights into the molecular interactions of thymoquinone with histone deacetylase: evaluation of the therapeutic intervention potential against breast cancer. Mol Biosyst. 2016 Jan;12(1):48-58. doi: 10.1039/c5mb00412h.

Pinchevsky Y, Butkow N, Raal FJ, Chirwa T, Rothberg A. Demographic and Clinical Factors Associated with Development of Type 2 Diabetes: A Review of the Literature. Int J Gen Med. 2020 Mar 31;13:121-129. doi: 10.2147/IJGM.S226010.

Radi R, Denicola A, Morgan B, Zielonka J. Foreword to the Free Radical Biology and Medicine Special Issue on ¨Current fluorescence and chemiluminescence approaches in free radical and redox biology¨. Free Radic Biol Med. 2018 Nov 20;128:1-2. doi: 10.1016/j.freeradbiomed.2018.09.027.

Rellmann Y, Eidhof E, Dreier R. Review: ER stress-induced cell death in osteoarthritic cartilage. Cell Signal. 2021 Feb;78:109880. doi: 10.1016/j.cellsig.2020.109880.

Ricobaraza A, Cuadrado-Tejedor M, Pérez-Mediavilla A, Frechilla D, Del Río J, García-Osta A. Phenylbutyrate ameliorates cognitive deficit and reduces tau pathology in an Alzheimer's disease mouse model. Neuropsychopharmacology. 2009 Jun;34(7):1721-32. doi: 10.1038/npp.2008.229.

Romao I, Roth J. Genetic and environmental interactions in obesity and type 2 diabetes. J Am Diet Assoc. 2008 Apr;108(4 Suppl 1):S24-8. doi: 10.1016/j.jada.2008.01.022.

Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol. 2007 Jul;8(7):519-29. doi: 10.1038/nrm2199.

Roscioni SS, Heerspink HJ, de Zeeuw D. The effect of RAAS blockade on the progression of diabetic nephropathy. Nat Rev Nephrol. 2014 Feb;10(2):77-87. doi: 10.1038/nrneph.2013.251.

Saeedi P, Salpea P, Karuranga S, Petersohn I, Malanda B, Gregg EW, Unwin N, Wild SH, Williams R. Mortality attributable to diabetes in 20–79 years old adults, 2019 estimates: Results from the International Diabetes Federation Diabetes Atlas. Diabetes research and clinical practice. 2020 Apr 1;162:108086.

Sagoo MK, Gnudi L. Diabetic Nephropathy: An Overview. Methods Mol Biol. 2020;2067:3-7. doi: 10.1007/978-1-4939-9841-8_1.

Sakao S, Tatsumi K, Voelkel NF. Endothelial cells and pulmonary arterial hypertension: apoptosis, proliferation, interaction and transdifferentiation. Respir Res. 2009 Oct 13;10(1):95. doi: 10.1186/1465-9921-10-95.

Scarpellini E, Rinninella E, Basilico M, Colomier E, Rasetti C, Larussa T, Santori P, Abenavoli L. From Pre- and Probiotics to Post-Biotics: A Narrative Review. Int J Environ Res Public Health. 2021 Dec 21;19(1):37. doi: 10.3390/ijerph19010037.

Senft D, Ronai ZA. UPR, autophagy, and mitochondria crosstalk underlies the ER stress response. Trends Biochem Sci. 2015 Mar;40(3):141-8. doi: 10.1016/j.tibs.2015.01.002

Sharma K, Ziyadeh FN. Biochemical events and cytokine interactions linking glucose metabolism to the development of diabetic nephropathy. Semin Nephrol. 1997 Mar;17(2):80-92. Erratum in: Semin Nephrol 1997 Jul;17(4):391.

Sies H, Berndt C, Jones DP. Oxidative Stress. Annu Rev Biochem. 2017 Jun 20;86:715-748. doi: 10.1146/annurev-biochem-061516-045037.

Staveness D, Bosque I, Stephenson CR. Free Radical Chemistry Enabled by Visible Light-Induced Electron Transfer. Acc Chem Res. 2016 Oct 18;49(10):2295-2306. doi: 10.1021/acs.accounts.6b00270.

Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet. 2005 Apr 9-15;365(9467):1333-46. doi: 10.1016/S0140-6736(05)61032-X.

Supraja A, Dinesh MG, Rajasekaran S, Balaji TM, Rao SR. Effect of Cyclosporin A and Angiotensin II on cytosolic calcium levels in primary human gingival fibroblasts. Dent Res J (Isfahan). 2016 Sep;13(5):405-412. doi: 10.4103/1735-3327.192276.

Sutendra G, Dromparis P, Wright P, Bonnet S, Haromy A, Hao Z, McMurtry MS, Michalak M, Vance JE, Sessa WC, Michelakis ED. The role of Nogo and the mitochondria-endoplasmic reticulum unit in pulmonary hypertension. Sci Transl Med. 2011 Jun 22;3(88):88ra55. doi: 10.1126/scitranslmed.3002194.

Tabas I. The role of endoplasmic reticulum stress in the progression of atherosclerosis. Circ Res. 2010 Oct 1;107(7):839-50. doi: 10.1161/CIRCRESAHA.110.224766.

Tang X, Rong G, Bu Y, Zhang S, Zhang M, Zhang J, Liang X. Advanced oxidation protein products induce hypertrophy and epithelial-to-mesenchymal transition in human proximal tubular cells through induction of endoplasmic reticulum stress. Cell Physiol Biochem. 2015;35(2):816-28. doi: 10.1159/000369740.

Tang Y, Zhou X, Cao T, Chen E, Li Y, Lei W, Hu Y, He B, Liu S. Endoplasmic Reticulum Stress and Oxidative Stress in Inflammatory Diseases. DNA Cell Biol. 2022 Nov;41(11):924-934. doi: 10.1089/dna.2022.0353.

Tarr, J.M.; Kaul, K.; Chopra, M.; Kohner, E.M.; Chibber, R. Pathophysiology of diabetic retinopathy. ISRN Ophthalmol. 2013, 2013, 1–13. [CrossRef]

Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, Ferrario F, Fogo AB, Haas M, de Heer E, Joh K, Noël LH, Radhakrishnan J, Seshan SV, Bajema IM, Bruijn JA; Renal Pathology Society. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol. 2010 Apr;21(4):556-63. doi: 10.1681/ASN.2010010010.

Torino MI, Font de Valdez G, Mozzi F. Biopolymers from lactic acid bacteria. Novel applications in foods and beverages. Front Microbiol. 2015 Sep 11;6:834. doi: 10.3389/fmicb.2015.00834.

Wang Z, Zheng S, Gu Y, Zhou L, Lin B, Liu W. 4-PBA Enhances Autophagy by Inhibiting Endoplasmic Reticulum Stress in Recombinant Human Beta Nerve Growth Factor-Induced PC12 cells After Mechanical Injury via PI3K/AKT/mTOR Signaling Pathway. World Neurosurg. 2020 Jun;138:e659-e664. doi: 10.1016/j.wneu.2020.03.038.

Wanner C, Inzucchi SE, Lachin JM, Fitchett D, von Eynatten M, Mattheus M, Johansen OE, Woerle HJ, Broedl UC, Zinman B; EMPA-REG OUTCOME Investigators. Empagliflozin and Progression of Kidney Disease in Type 2 Diabetes. N Engl J Med. 2016 Jul 28;375(4):323-34. doi: 10.1056/NEJMoa1515920.

World Health Organization (WHO). Global Report on Diabetes; WHO: Geneva, Switzerland, 2017. Available online: http://www. who.int/diabetes/global-report/en/ (accessed on 22 September 2018)

Xiao C, Giacca A, Lewis GF. Sodium phenylbutyrate, a drug with known capacity to reduce endoplasmic reticulum stress, partially alleviates lipid-induced insulin resistance and beta-cell dysfunction in humans. Diabetes. 2011 Mar;60(3):918-24. doi: 10.2337/db10-1433..

Yan Q, Li D, Jia S, Yang J, Ma J. Novel gene-based therapeutic approaches for the management of hepatic complications in diabetes: Reviewing recent advances. Journal of Diabetes and its Complications. 2024 Jan 11:108688.

Yang WH, Deng YT, Hsieh YP, Wu KJ, Kuo MY. NADPH Oxidase 4 Mediates TGFβ1-induced CCN2 in Gingival Fibroblasts. J Dent Res. 2015 Jul;94(7):976-82. doi: 10.1177/0022034515580986.

Yang, B.; Hodgkinson, A.; Millward, B.A.; Demaine, A.G. High glucose-induced DNA-binding activities of nuclear factor of activated T cells 5 and carbohydrate response element binding protein to the myo-inositol oxygenase gene are inhibited by sorbinil in peripheral blood mononuclear cells from patients with type 1 diabetes mellitus and nephropathy. Int. J. Diabetes Mellit. 2010

Yaribeygi H, Sathyapalan T, Atkin SL, Sahebkar A. Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus. Oxid Med Cell Longev. 2020 Mar 9;2020:8609213. doi: 10.1155/2020/8609213.

Yeager ME, Reddy MB, Nguyen CM, Colvin KL, Ivy DD, Stenmark KR. Activation of the unfolded protein response is associated with pulmonary hypertension. Pulm Circ. 2012 Apr-Jun;2(2):229-40. doi: 10.4103/2045-8932.97613.

Yoshino H, Kumai Y, Kashiwakura I. Effects of endoplasmic reticulum stress on apoptosis induction in radioresistant macrophages. Mol Med Rep. 2017 May;15(5):2867-2872. doi: 10.3892/mmr.2017.6298.

Zeng Y, Zhao H, Zhang T, Zhang C, He Y, Du L, Zuo F, Wang W. Lung-protective effect of Punicalagin on LPS-induced acute lung injury in mice. Biosci Rep. 2022 Jan 28;42(1):BSR20212196. doi: 10.1042/BSR20212196.

Zhang H, Colclough K, Gloyn AL, Pollin TI. Monogenic diabetes: a gateway to precision medicine in diabetes. J Clin Invest. 2021 Feb 1;131(3):e142244. doi: 10.1172/JCI142244.

Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol. 2018 Feb;14(2):88-98. doi: 10.1038/nrendo.2017.151.

Zhou F, Zhang W, Zhou J, Li M, Zhong F, Zhang Y, Liu Y, Wang Y. Involvement of endoplasmic reticulum stress in formalin-induced pain is attenuated by 4-phenylbutyric acid. J Pain Res. 2017 Mar 20;10:653-662. doi: 10.2147/JPR.S125805.

Zhou X, Xu Y, Gu Y, Sun M. 4-Phenylbutyric acid protects islet β cell against cellular damage induced by glucocorticoids. Mol Biol Rep. 2021 Feb;48(2):1659-1665. doi: 10.1007/s11033-021-06211-5.