



REPUBLIC OF TÜRKİYE
MARMARA UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

**EVALUATION OF THE EFFICACY OF DIPEPTIDYL PEPTIDASE-4
INHIBITOR AND SODIUM-GLUCOSE COTRANSPORTER-2
INHIBITOR IN THE DIABETES-INDUCED ALZHEIMER'S MODEL**

MOHAMMED ABDO QASEM RADMAN KHALED
MASTER'S THESIS

SUPERVISOR
ASST. PROF. AYŞE NUR YAVUZ
MASTER'S PROGRAM IN PHARMACOLOGY

İSTANBUL-2025



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DECLARATION

I hereby declare that this thesis is entirely my own original work. I affirm that I have not engaged in any unethical conduct at any stage, from planning to writing the thesis. Academic and ethical standards have been followed to obtain all the information and data presented in this study. I have properly cited all sources for any information, ideas, or interpretations not directly derived from this research, and all such references have been included in the bibliography. I also confirm that I have not violated any patent or copyright laws during the preparation and writing of this thesis.



Mohammed Abdo Qasem Radman Khaled

DEDICATION

To the soul of my beloved mother, she was the reason for my existence and the light of my life. I had always hoped she would live to witness this achievement and share in its joy. May Allah have mercy on her soul and accept this work as part of her legacy.

To my esteemed father, my first supporter and the crown upon my head, Who instilled in me a love for knowledge and gave generously of his time and effort, to him, I extend my deepest gratitude and sincere appreciation.

To my dear brothers and sisters, who have been, and continue to be, a steadfast source of support and strength throughout my journey.

To my respected professors, whose guidance and academic mentorship have illuminated my path and enriched my understanding.

And to all those who supported me, even with a kind word, I dedicate this humble work in recognition of your kindness and gratitude for your encouragement.

Mohammed Abdo Qasem Radman Khaled

ACKNOWLEDGMENTS

I would like to begin by expressing my sincere gratitude to my esteemed supervisor, Asst. Prof. Ayşe Nur Yavuz, for her exceptional guidance, unwavering support, and invaluable insights throughout this research. Her dedication and belief in my potential have had a profound impact on both my academic journey and personal growth.

I am deeply thankful to the distinguished faculty members of the Department of Pharmacology, Prof. Dr. Hatice Kübra Elçioğlu, Prof. Dr. Levent Kabasakal, and Assoc. Prof. Büşra Ertaş, and Assoc. Prof. Muhammet Emin Çam, for their academic contributions, insightful advice, and unwavering support, which significantly enriched my research and academic journey.

I extend my heartfelt thanks to my dear friends, Cihat Yıldız and Kadir Demirci, staff members at the Experimental Animal Research and Application Center of Marmara University, for their constant support throughout all stages of my thesis work.

I would also like to sincerely thank Assoc. Prof. Özlem Tuğçe Çilingir Kaya for their invaluable assistance with the histological analyses that greatly contributed to the success of this study.

I am deeply grateful to my father, my brothers, and especially my sisters Nadia and Sabreen, for their unwavering love and support, which sustained me throughout my academic journey.

I sincerely thank my dear friend Zaid Alhaj for his unwavering moral support throughout my master's studies.

I would also like to thank all my friends from my undergraduate and master's degree studies for their companionship and support. Your presence made this academic journey far more meaningful and enjoyable.

This research was financially supported by the Marmara University Scientific Research Projects Committee under the project number TYL-2024-11371

Mohammed Abdo Qasem Radman Khaled

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LIST OF ABBREVIATIONS AND SYMBOLS

AD	: Alzheimer's Disease
ADA	: American Diabetes Association
ADRDA	: Alzheimer's Disease and Related Disorders Association
AKT kinase	: Protein kinase B (AKT)
AMPK	: AMP-Activated Protein Kinase
AMPK/BDNF1/P70SK2	: AMP-Activated Protein Kinase / Brain-Derived Neurotrophic Factor / Ribosomal Protein S6 Kinase Beta- 1
ApoE4	: Apolipoprotein E4
APP	: Amyloid precursor protein
ARIA-E	: Amyloid-Related Imaging Abnormalities – Edema
ASOs	: Antisense oligonucleotides
ATP	: Adenosine Triphosphate
A β	: Amyloid beta
BACE1	: Beta-Site Amyloid Precursor Protein Cleaving Enzyme 1
BDNF	: Brain-Derived Neurotrophic Factor
BBB	: Blood-brain barrier
BuChE	: Butyrylcholinesterase
CDA	: Canadian Diabetes Association
Camp	: Cyclic Adenosine Monophosphate
CNS	: Central nervous system
CGMP	: Cyclic guanosine monophosphate
ChEIs	: Cholinesterase inhibitors
ChAT	: Choline Acetyltransferase enzyme

DKA	: Diabetic ketoacidosis
DPP-IV	: Dipeptidyl Peptidase-4
DPP-4is	: Dipeptidyl peptidase 4 inhibitors
EOAD	: Early-onset Alzheimer's Disease
FDA	: Food and Drug Administration
GABAergic	: GABA (Gamma-Aminobutyric Acid)
GDM	: Gestational diabetes mellitus
GLP-1	: Glucagon-like peptide-1
GLP-1/GLP-1R	: Glucagon-Like Peptide-1 / Glucagon-Like Peptide-1 Receptor
GSK3 β	: Glycogen Synthase Kinase 3 Beta
GSH	: Glutathione
HbA1c	: Hemoglobin A1c
HFD	: High-fat diet
HFrEF	: Heart failure with reduced ejection fraction
HLA	: Human Leukocyte Antigen gene
HNF1	: Hepatocyte Nuclear Factor 1
HPA	: Hypothalamic-Pituitary-Adrenal axis
IDF	: International Diabetes Federation
IDE	: Insulin-degrading enzyme
IDOL/PCSK9-LDLR-Apo	: Inducible Degradation of the Low-Density Lipoprotein Receptor / Proprotein Convertase Subtilisin/Kexin Type 9 - Low-Density Lipoprotein Receptor - Apolipoprotein E
IGF	: Insulin-like growth factor
IWG	: International Working Group
JNK	: c-Jun N-terminal kinase
KATP	: ATP-sensitive Potassium channels

LADA	: Latent autoimmune diabetes in adults
LDLR	: Low-Density Lipoprotein Receptor
LOAD	: Late-onset Alzheimer's Disease
LRP-1	: Low-Density Lipoprotein Receptor-Related Protein1
MAP	: Microtubule-associated protein
MCI	: Mild cognitive impairment
MODY	: Maturity onset diabetes of the young
MODY2	: Maturity onset diabetes of the young, type 2
MODY3	: Maturity onset diabetes of the young, type 3
MRI	: Magnetic resonance imaging
Mtor	: Mechanistic Target of Rapamycin
NIA-AA	: National Institute on Aging and the Alzheimer's Association
NINCDS	: National Institute of Neurological and Communicative Disorders and Stroke
NFTs	: Neurofibrillary tangles
NLRP3	: NOD-Like Receptor Family Pyrin Domain Containing 3
NMDA	: N-Methyl-D-Aspartate receptor
NSAIDs	: Non-steroidal anti-inflammatory Drugs
OGTT	: Oral glucose tolerance test
PD	: Parkinson's disease
PDEs	: Phosphodiesterases
PI3K	: Phosphoinositide 3-kinase
PI3K-AKT	: Phosphoinositide 3-kinase - Protein Kinase B pathway
PPAR	: Peroxisome Proliferator-Activated Receptor
PPAR- γ	: Peroxisome Proliferator-Activated Receptor Gamma

PSEN1	: Presenilin 1
PSEN2	: Presenilin 2
RAGE	: Receptor for Advanced Glycation End-products
RAS	: Renin-Angiotensin System
RXR	: Retinoid X receptor
SGLTIs	: Sodium-Glucose Cotransporter Inhibitors
SOD	: Superoxide Dismutase
SPECT	: Single-Photon Emission Computed Tomography
SUR1	: Sulfonylurea Receptor 1
Sus	: Sulphonylureas
T1DM	: Type 1 diabetes mellitus
T2DM	: Type 2 diabetes mellitus
TRPM4	: Transient Receptor Potential Melastatin 4
TREM2	: Triggering Receptor Expressed on Myeloid cells 2
TZDs	: Thiazolidinediones
WHO	: The World Health Organization
ZDF	: Zucker diabetic fatty
α -secretase	: α -secretase
β -secretase	: β -secretase
γ -secretase	: γ -secretase

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1. ÖZET

Tez Başlığı: Dipeptidil Peptidaz-4 İnhibitörü ve Sodyum-Glukoz Ortak Taşıyıcı-2 İnhibitörünün Diyabetle İndüklenen Alzheimer Modelinde Etkinliklerinin Değerlendirilmesi

Öğrenci Adı, Soyadı: Mohammed Abdo Qasem Radman Khaled

Danışman Adı: Dr. Öğr. Üyesi Ayşe Nur Yavuz

Program Adı: Farmakoloji Yüksek Lisans Programı

Amaç: Bu tezde, empagliflozin ve vildagliptin'in hem ayrı ayrı hem de birlikte uygulandıklarında, Tip 2 Diabetes Mellitus (T2DM)'ye bağlı oluşan Alzheimer hastalığı (AH) sıçan modelindeki nöroprotektif etkileri ve potansiyel mekanizmalarının değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmamızda kırk Sprague-Dawley sıçanı kontrol ve AH gruplarına ayrılmıştır. T2DM, dört haftalık yüksek yağlı diyet (YYD)'in ardından düşük doz streptozotosin (STZ) uygulamasıyla oluşturulmuştur. Tedavi gruplarına son dört hafta boyunca sırasıyla Empagliflozin, Vildagliptin ve her ikisi birlikte uygulanmıştır. Sıçanların diyabetik durumları haftalık kan glukoz ölçümü ve oral glukoz testi ile değerlendirilirken; bilişsel performansları; açık alan, yeni nesne tanıma, pasif kaçınma ve Morris su tankı testleriyle değerlendirilmiştir.

Bulgular: STZ uygulaması sonrası vücut ağırlıkları gruplar arasında benzer bulunmuştur. AH modeli sıçanlarda belirgin hiperglisemi ve glukoz intoleransı gözlenmiş; vildagliptin ile kısmen iyileşme, empagliflozin ile daha belirgin düzeltme sağlanmış ve kombinasyon tedavisi ile glukoz düzeyleri tam olarak normal düzeye dönmüştür. AH grubundaki anksiyete benzeri davranış ve tanıma belleği bozuklukları her bir monoterapi ile kısmen gerilerken, kombinasyon tedavisi ile tamamen düzeltilmiştir. Pasif kaçınma ve mekânsal öğrenme bozuklukları da benzer bir seyir izlemiş ve kombinasyon tedavisi en yüksek fayda sağlanmıştır.

Sonuç: Dual DPP-4/SGLT2 inhibisyonu, YYD+düşük doz STZ sıçan modelinde glisemiye en etkili şekilde normalize etmiş ve tanıma belleği, kısa süreli ve mekânsal öğrenmeyi ilaçların tek başlarına uygulanmalarına kıyasla daha iyi düzelterek sinerjistik nöroprotektif potansiyeli ortaya koymuştur. Bu bulgular, T2DM'ye bağlı bilişsel gerileme için kombine tedavinin mekanistik ve translasyonel çalışmalarını haklı çıkarmaktadır.

Anahtar Kelimeler: Alzheimer hastalığı, Tip 2 diyabet mellitus, Sodyum-glukoz kotransporter-2 inhibitörü, Dipeptidil peptidaz-4 inhibitörü.

2. SUMMARY

Title of Thesis: Evaluation of the Efficacy of Dipeptidyl Peptidase-4 Inhibitor and Sodium-Glucose Cotransporter-2 Inhibitor in the Diabetes-Induced Alzheimer's Model

Student Name, Surname: Mohammed Abdo Qasem Radman Khaled

Supervisor Name: Asst. Prof. Ayşe Nur Yavuz

Program Name: Pharmacology Master's Program

Objective: In this thesis, it was aimed to evaluate the neuroprotective effects and potential mechanisms of empagliflozin and vildagliptin, both individually and in combination, in a rat model of Type-2 Diabetes Mellitus (T2DM)-induced Alzheimer's Disease (AD).

Material and Methods: In our study, forty Sprague-Dawley rats were divided into control and experimental (AH) groups. T2DM was induced by a low-dose streptozotocin (STZ) injection following four weeks of high-fat-diet (HFD) feeding. The treatment groups received Empagliflozin, Vildagliptin, or a combination of both drugs for last four weeks. The diabetic status of the rats was monitored through weekly blood glucose measurements and oral glucose tolerance tests. Cognitive performance was assessed using the open field, novel object recognition, passive avoidance, and Morris' water maze tests.

Results: Body weight was similar across groups post-STZ. AH rats showed marked hyperglycemia and glucose intolerance, modestly improved by vildagliptin, more by empagliflozin, and fully normalized by the combination. Anxiety-like behavior and recognition-memory deficits in AH animals were partially reversed by each monotherapy and fully rescued by combination treatment. Passive avoidance and spatial learning impairments followed the same pattern, with dual therapy yielding the greatest benefit.

Conclusion: Dual DPP-4/SGLT2 inhibition in the T2DM-induced AD rat model most effectively normalized glycemia and rescued anxiety-like behavior, recognition memory, and spatial learning versus single agents, highlighting synergistic neuroprotective potential. These findings justify further mechanistic and translational studies of combined therapy for diabetes-related cognitive decline.

Keywords : Alzheimer's disease, Type 2 diabetes mellitus, Sodium-glucose cotransporter-2 inhibitor, Dipeptidyl peptidase-4 inhibitor.

3. INTRODUCTION AND OBJECTIVE

Type 2 Diabetes Mellitus (T2DM) and Alzheimer's Disease (AD) are prevalent chronic diseases that pose major public health challenges worldwide. T2DM is a metabolic condition of persistent hyperglycemia caused by either resistance to or insufficiency of insulin. In contrast, AD is a neurodegenerative disorder that mainly targets the cognitive function, especially that concerned with memory and learning. Recent studies have emphasized the bidirectional link between T2DM and AD, with T2DM patients being at significantly high risk of developing AD. The association implies a complex pathophysiological overlap of metabolic pathology with neurodegenerative processes (Motaghi et al., 2024; Gomez-Coral, 2024).

Various mechanisms linking T2DM to AD have been identified in recent research, such as insulin resistance, amyloid- β (A β) deposition, oxidative stress, neuroinflammatory pathways, and disruption of mitochondrial function (Lynn et al., 2022). Another feature of T2DM, insulin resistance, extends beyond peripheral tissues to influence cerebral functions as well, thereby promoting cognitive impairment and enhancing the development of neurodegenerative disorders, particularly AD. Further, the altered metabolic state of T2DM aggravates amyloid plaque deposition and neurofibrillary tangle (NFT) formation, key pathological features of AD (Gomez-Coral, 2024). The interconnectedness of T2DM and AD renders diagnosis and treatment difficult, and hence the necessity to explore novel therapeutic strategies with concurrent treatment of these diseases.

Due to the complexity of these diseases, interest in repurposing existing pharmacological agents for the treatment of their double burden has seen a spike. Some of the most promising among these are Sodium-Glucose Cotransporter-2 (SGLT-2) inhibitors and dipeptidyl peptidase 4 inhibitors (DPP-4I's), two drug classes primarily developed for the management of T2DM. Empagliflozin, a selective SGLT-2 inhibitor, reduces blood glucose levels by inhibiting the reabsorption of glucose in the kidney and thereby increasing the urinary excretion of glucose. In addition to its glucose-lowering action, empagliflozin has manifested cardioprotective and renoprotective effects, with a particular focus on whether it has neuroprotective effects (Siamashvili & Davis, 2021; Tzoulis, 2024).

Similarly, vildagliptin, which is a DPP-4 inhibitor, stimulates insulin secretion and suppresses glucagon secretion by modulating incretin hormones, improving glucose homeostasis, and stimulating insulin sensitivity (Edward Foley, 2014). Both preclinical and clinical models also revealed that SGLT-2 and DPP-4 inhibitors can have beneficial effects on the brain and prevent

or even reverse AD development in T2DM patients (Hierro-Bujalance et al., 2020). They are reported to influence several biochemical processes like neuroinflammation, oxidative stress, and A β deposition that are main processes implicated in the pathogenesis of AD (Hierro-Bujalance & Garcia-Alloza, 2023; Pawlos et al., 2021).

Despite these encouraging results, the neuroprotective actions of SGLT-2 and DPP-4 inhibitors remain incompletely understood. Whether they can be employed in treating AD when there is also T2DM is not yet clearly established. Neuroinflammation and oxidative stress are significant contributors to the pathogenesis of both conditions, and inhibition of these activities could have important therapeutic benefits. SGLT-2 inhibitors like empagliflozin have also been found to decrease oxidative stress and inflammation in tissues, including the brain (Sim et al., 2021). Similarly, DPP-4 inhibitors like vildagliptin have also been found to have anti-inflammatory actions, which may be beneficial in minimizing the neurodegenerative processes of AD (Kosaraju et al., 2013). Furthermore, both compound classes were found to modulate A β accumulation and tau phosphorylation, pathophysiologic processes at the core of AD development (Chouhan et al., 2025).

The potential therapeutic combination of SGLT-2 and DPP-4 inhibitors is a promising situation. Although the agents target disparate mechanisms, their concurrent use may enhance neuroprotective effects, providing a more integrated approach to managing metabolic and cognitive dysfunction in patients with T2DM. The combined effect of these two drug classes in T2DM-related AD has not been thoroughly investigated. This absence of data highlights the necessity to explore the synergistic therapeutic efficacy in preclinical models (Sim et al., 2021; Sim et al., 2023).

This study aimed to evaluate the neuroprotective effects and potential mechanisms of empagliflozin and vildagliptin, both individually and in combination, in a rat model of T2DM-induced AD. The study assessed the impact of these drugs on cognitive function, as well as on biochemical markers related to neurodegeneration, insulin resistance, and A β accumulation. The rat model employed in this research mimics the pathological features of both T2DM and AD, providing a valuable platform for examining the effects of these drugs on cognitive performance and neuroinflammation. A series of behavioral assays, including the Open Field Test (OFT), Morris Water Maze (MWM), Novel Object Recognition (NOR), and Passive Avoidance (PA) test, were utilized to assess different aspects of memory and learning (Nunez, 2008; Vila, 2023).

Biochemical markers of interest included insulin levels, A β (A β 1-42), tau phosphorylation, acetylcholinesterase (AChE) activity, SGLT-2 expression, and glycogen synthase kinase 3 beta (GSK-3 β). Insulin and A β levels serve as indicators of metabolic and neurodegenerative alterations associated with T2DM and AD, while tau phosphorylation, AChE activity, and GSK-3 β activity are key in the development of cognitive deficits and NFTs (Farooqui, 2013; Mullins et al., 2017).

In the present experimental protocol, diabetic rats were treated with empagliflozin at a dose of 10 mg/kg/day and vildagliptin at 4 mg/kg/day for eight weeks. The primary aim was to determine whether combined therapy provides greater neuroprotective effects than monotherapy with each drug separately. The study also aimed to assess the mechanisms by which these drugs influence brain pathology, in this case, insulin resistance, neuroinflammation, and A β deposition.

The potential study implications are extensive. Successful completion will open the door for clinical trials assessing the application of SGLT-2 and DPP-4 inhibitors in T2DM and AD patients. Further, this study can lead to the development of novel therapeutic interventions for the treatment of cognitive impairment in diabetic patients, which will eventually enhance the quality of life of individuals suffering from both diseases. Furthermore, it may yield novel insights into the intertwined pathophysiology of T2DM and AD and enhance understanding of possible interventions to prevent or attenuate these disabling disorders.

4. BACKGROUND

4.1. Diabetes Mellitus (DM)

4.1.1. General information about DM

Diabetes Mellitus (DM) is a chronic metabolic disorder induced by elevated blood glucose levels due to either a lack of insulin secretion or an impaired function of this hormone. These disturbances may lead to severe health complications and decreased quality of life, affecting multiple organ systems (Sun et al., 2023). According to the International Diabetes Federation (IDF), 463 million adults worldwide were suffering from diabetes in 2019. The future estimates are a staggering rise of seven hundred million individuals likely to be affected by 2045 if effective prevention strategies are not initiated (Sugandh et al., 2023; Yedjou et al., 2023).

The increasing incidence and complications of DM are responsible in large measure for morbidity, mortality, and economic costs. DM and its complications have been put at an estimated cost of about US\$500 billion of worldwide healthcare spending every year, without including indirect costs like productivity loss (Bempah, 2021). The disease impacts essential metabolic processes and pathological mechanisms, thereby inhibiting the wound healing process in patients with the disease (Bempah, 2021).

4.1.2. Pathophysiology of DM

Insulin is an essential hormone that plays a key role in the cellular metabolism of glucose, lipids, and amino acids. Following food intake, insulin promotes glucose entry into cells for eventual utilization as energy or storage as glycogen and enhances absorption and retention of fatty acids and amino acids (De la Cruz Concepción et al., 2023). Insulin is secreted by pancreatic β -cells in response to high blood glucose levels, which subsequently acts on target cell surface receptors. By this action, intracellular signaling pathways are initiated to increase the activity of glucose transporter proteins. Deficiency of insulin initiates a catabolic state of increased rates of lipolysis and glycogenolysis, ultimately resulting in hyperglycemia, the signature of diabetes (Veli & Peltzer, 2023).

Type 1 DM (T1DM) is caused by autoimmune pancreatic β -cell destruction with absolute insulin deficiency, causing hyperglycemia and, in some cases, diabetic ketoacidosis. Type 2 DM (T2DM) results from a multigenic interaction of environmental factors and genetic susceptibility, causing insulin resistance and gradual β -cell loss. The β -cells initially react to insulin resistance by elevating the secretion of insulin; however, chronic stress has the potential

to cause β -cell dysfunction and depletion, ultimately leading to diabetes development (Siddiqui et al., 2023).

The pathogenesis of T2DM is mediated through mechanisms involving β -cell apoptosis, glucotoxicity, lipotoxicity, and congenital genetic defects. Central obesity and insulin resistance are closely related and reduce the responsiveness of the β -cells to the need for insulin. Up to 50% of the β -cells' functional capacity to secrete insulin may be lost at the time of diagnosis. Chronic hyperglycemia has been linked with numerous complications, including macrovascular, microvascular, and neurological entities. The complex interaction between insulin signaling, genetics, and metabolic dysregulation must be well understood to unveil the pathophysiology of diabetes and implement effective therapies (Yang et al., 2023; Carlberg et al., 2023).

4.1.3. Classification of DM

The new classification developed in 1997 categorizes diabetes into four groups: T1DM, T2DM, other specific types, and gestational diabetes (Yash Sahebrao Chaudhari et al., 2023).

T1DM is an autoimmune disease, results from the destruction of pancreatic islet cells and usually develops in childhood or early adulthood. T1DM leads to absolute insulin deficiency and may result in complications such as ketoacidosis. Biochemical investigations reveal hyperglycemia with low C-peptide levels, indicating a deficiency of insulin production. T1DM, which comprises 5-10% of the cases, is common in Scandinavia, where it is affected by genetic components such as the HLA gene on chromosome 6 and environmental factors such as specific viruses. There are subtypes of T1DM that involve differences in autoimmunity and insulin secretion. Conversely, autoimmune Type 1A DM is linked to other autoimmune disorders, including Addison's disease and Hashimoto's thyroiditis, and is induced by aspects such as viral infections and cow's milk protein (Zajec et al., 2022; Bereda, 2022).

T2DM is a common disorder that results from glucose control deficiency caused by dysfunctional pancreatic beta cells and insulin resistance. Typically diagnosed after the age of 40, it is responsible for 90% of diabetes globally, with an increasing incidence with advancing age. T2DM has two subgroups: obesity with diabetes, which is defined by insulin resistance due to changes in cell receptors and central fat distribution, and non-obese T2DM, which is defined by insulin resistance and a defect in insulin secretion. Accounting for 90-95% of cases worldwide, T2DM is associated with etiologies like obesity, sedentary lifestyle, and diet. It's characterized by insulin resistance leading to elevated blood glucose levels and is caused by

lifestyle, genetics, environmental toxins, and obesity. Proper diet and physical activity are the most significant preventive measures (Zeliger, 2023; Yash Sahebrao Chaudhari et al., 2023).

Gestational DM (GDM) is a temporary diabetes in pregnancy, causing insulin resistance and mother-fetal risk. It is found in 1-14% of pregnancies, differing from T2DM, with risk factors including obesity and family history. Its complications are hypertension, pre-eclampsia, diabetic retinopathy, and increased birth injury risks. Maintaining optimal glycemic control is key in avoiding complications, while postpartum care plays a key role in avoiding the development of future T2DM. Pregnancy complicated by diabetes carries higher risks for severe congenital malformations, highlighting the need for optimal glucose control during pregnancy (Barbach et al., 2023; Zygmunt-Siembida et al., 2023).

Maturity-onset diabetes of the young (MODY) is a genetic defect in beta cell function, typically occurring before the age of 25. The disease, which is typified by little or no complications in insulin activity, can be diagnosed through family medical history. MODY has numerous forms, and among the most frequent are MODY3 (HNF1-alpha defect) and MODY2 (glucokinase defect). Latent autoimmune diabetes in adults (LADA) is an adult-onset diabetes that takes place in 2-12% of adults and is liable to be mistaken for T2DM. It requires treatment with insulin earlier compared to T2DM (Younis et al., 2022).

4.1.4. Diagnostic criteria

The World Health Organization (WHO), the American Diabetes Association (ADA), and the Canadian Diabetes Association (CDA) have established diagnostic criteria for diabetes, such as fasting plasma glucose, 2-hour plasma glucose, a glucose load, and random plasma glucose. Tests used for diagnosing T2DM comprise the measurement of short-term and long-term glucose levels. Short-term measurement includes fasting blood glucose, two-hour blood glucose following an oral glucose tolerance test (OGTT), and random blood glucose. Long-term measurement uses Hemoglobin A1c (HbA1c), which is the mean glucose level of the last 2-3 months (Khamis, 2023).

The diagnostic criteria are a fasting plasma glucose level of 126 mg/dL or more, a 2-hour plasma glucose level of 200 mg/dL or more following a glucose challenge, or a random plasma glucose level of 200 mg/dL or more. HbA1c is approved by the ADA as a test for diagnosis with a cutoff value of 6.5%. HbA1c is presented as a percentage, with elevated values pointing to chronically elevated glucose levels (Chen et al., 2023).

4.1.5. Complications of DM

Diabetes complications consist of microvascular and macrovascular complications. Microvascular complications, which result from hyperglycemia, affect organs like the eyes, nerves, and kidneys. Those affecting the nerves, such as diabetic neuropathy, are found in two out of three individuals with diabetes, causing abnormal sensations, numbness, and potential complications like ulcers and amputations. Diabetic nephropathy, which is common in diabetes, results in high albumin in urine and is one of the causes of end-stage renal disease (Wilson, 2023). Management is with angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) to control blood pressure and slow kidney damage. Macrovascular complications comprise cardiovascular diseases, which have a more rapid progression in diabetics. Diabetes is the leading cause of visual impairment, being associated with complications such as retinopathy and also with an increased occurrence of glaucoma and cataract development (Sharifan et al., 2023).

Diabetic ketoacidosis (DKA), predominantly occurring in T1DM, is due to a deficiency of insulin, leading to dehydration, electrolyte imbalance, and acidosis. Meticulous fluid and electrolyte management in the intensive care unit setting is essential during DKA management. Generally, maintenance of blood glucose levels within normal limits is paramount to preventing complications, with tight monitoring of glycemic levels, regulation of blood pressure, and constant monitoring (Healy et al., 2023).

4.2. Alzheimer's Disease (AD)

4.2.1. General information about AD

Alzheimer's Disease (AD) is the most common cause of dementia related to aging and is a progressive neurodegenerative disorder that currently affects an estimated fifty million individuals worldwide, with an estimated 70% increase by the year 2050. It is a disorder that is characterized by cognitive impairment, memory loss, and functional deficits that are beyond those observed in normal aging; the progression of AD follows a sequence of stages that begin with a preclinical phase without obvious symptoms, advance to mild cognitive impairment (MCI), and then progress to dementia (Swapna et al., 2023; Patel, 2023).

In clinical practice, AD is primarily categorized as two broad types: autosomal dominant early-onset AD (EOAD) and sporadic late-onset AD (LOAD). Although LOAD is almost exclusively found in the elderly and shows a robust association with aging, it is also influenced by environmental determinants and genetic susceptibility, e.g., the presence of the ApoE4 allele. Conversely, the EOAD, although much rarer, is associated with disease-causing mutations in

distinct genes, including amyloid precursor protein (APP), presenilin proteins (PSEN1 and PSEN2). More precisely, mutations in the APP gene account for approximately 10–15% of individuals with EOAD, whereas mutations in PSEN1 account for approximately 30–70%, and in PSEN2 for less than 5% (Caldwell et al., 2022).

The pathogenesis of AD is primarily due to the abnormal metabolism of the APP in conjunction with the role of presenilin proteins (PSEN1 and PSEN2), together ensuring the production of the neurotoxic A β peptide. This deleterious sequence of events plays a vital role in inducing the disruption of synaptic function and neuronal loss. AD is one of the major causes of disability among the elderly, highlighting the imperative need to decipher its intricate, multifactorial etiology. A comprehensive knowledge of genetic determinants coupled with environmental determinants is critical for formulating effective preventive and therapeutic strategies (Ma et al., 2023).

4.2.2. Prevalence and incidence of AD

Recent trends show a significant shift in the incidence and prevalence of dementia in the broad spectrum of socioeconomic populations. Meta-analytic reviews exhibit decreased incidence of dementia in Western and developed countries, which is linked to better early life circumstances, higher levels of education, and improved management of cardiovascular risk factors. Low- and middle-income countries, however, might still witness increasing incidence rates, indicating the differences in the availability of healthcare and public health infrastructures. Surprisingly, the prevalence of MCI has largely remained stable or experienced minimal decreases in the past few decades (Brück et al., 2022).

AD is geographically distributed, with higher prevalence in Europe than in North America and lower prevalence generally in developing countries. The prevalence of dementia is age-dependent, with about 1.7% of those in their 65–69 years being affected. Although global prevalence has been noted to plateau, recent data bring conflicting trends, with some demonstrating decreases, whereas others report steady or increasing rates. More critically, age-specific trends for dementia incidence, particularly in those ≥ 85 years, are less clear (Lanctôt et al., 2023).

Lifestyle modifications such as smoking cessation, increased levels of physical activity, and weight loss have also shown promise in decreasing dementia risk, particularly in Western populations. Meanwhile, regulatory bodies like the FDA are evaluating emerging therapeutics aimed at decelerating cognitive decline in AD. Nevertheless, with the global dementia burden

projected to rise, there is a pressing need for coordinated governmental strategies to address its profound social and economic consequences (Arora et al., 2023; Chowdhary et al., 2022).

4.2.3. Etiology and epidemiology of AD

Epidemiological research indicates a lower global burden of AD in low- and middle-income countries, with marked heterogeneity between regions, as high rates have been described in Chinese urban populations and certain population groups in Latin America. Such disparities attest to the heterogeneity and sophistication of worldwide patterns related to AD. Quality AD care prioritizes patient health despite complicated symptoms, since growing recognition of the impact of environmental and behavioral factors on initiating and promoting disease state renders physical health a cornerstone of cognitive resilience in AD patients, who otherwise are vulnerable to comorbidities. In spite of this difficulty, the definitive diagnosis remains an intricate process, especially in the early phases, and hence, continues the efforts to detect trustworthy, non-invasive biomarkers for early detection (Huma et al., 2022).

The development of AD is due to a complex interaction between genetic predisposition and environmental factors, with advancing age being the strongest risk factor. The categorization of AD is generally based on parameters like the age of onset of symptoms, family history in terms of inheritance, and detection of pathogenic gene mutations. Continuing controversies over the amyloid cascade hypothesis revolve around mutations in the gene for the APP and in the presenilin genes (PSEN1 and PSEN2). Additional risk-associated genes, such as TREM2 and ApoE, have also been found to play a role in disease susceptibility (Gauvrit et al., 2022). Beyond genetics, systemic inflammation, vascular dysfunction, dietary deficiencies, obesity, T2DM, and environmental exposures all contribute to AD risk. Neuropathologically, AD is marked by progressive synaptic loss, neuronal degeneration, and atrophy of the hippocampus. Patients with Down syndrome are highly vulnerable to EOAD, basically due to overexpression of amyloidogenic proteins and simultaneous development of tau pathology (Ezkurdia et al., 2023).

4.2.4. Diagnostic criteria in AD

Clinical diagnosis of AD relies on cognitive domain and functional impairment evaluation, with biomarkers playing a critical role in raising diagnostic specificity. Different criteria (e.g., guidelines from the National Institute on Aging and Alzheimer's Association (NIA-AA) or the International Working Group (IWG)) require cognitive impairment, elimination of alternative etiologies, and biomarker evidence for AD pathology (Dang et al., 2023). Biomarkers, including cerebrospinal fluid, blood, and neuroimaging measures, allow for early detection.

Current NINCDS-ADRDA criteria, used since 1984, combine clinical and neuropathological characteristics; however, it is known that the range of AD is broader, requiring ongoing updates to the criteria. Inherited mutations with an autosomal dominant pattern are diagnostic hallmarks, and future criteria revisions will most likely incorporate biomarkers to increase diagnostic specificity (Dulewicz et al., 2022). Diagnostic criteria for potential AD dementia include insidious onset, cognitive decline, and impairment in cognitive domains.

Neuroimaging studies, including magnetic resonance imaging (MRI), are recommended to rule out the existence of other etiologies or coexisting diseases. A probable diagnosis of AD must be precluded if there is any evidence of significant cerebrovascular pathology, dementia with Lewy bodies, frontotemporal dementia, primary progressive aphasia, or the existence of underlying concurrent active pathologies or pharmacotherapies that affect cognitive function. This diagnostic approach enables the recognition of individuals with the highest likelihood of developing AD and emphasizes the increasing role of biomarkers in enhancing diagnostic accuracy (Zhang et al., 2023; Alsaraira et al., 2023).

4.2.5. Neuropathophysiology of AD

A number of hypotheses have been proposed to explain the complex pathophysiology of AD, including the amyloid cascade, tau protein propagation, neuroinflammation, mitochondrial dysfunction, and oxidative stress (Nasb, 2024). Among them, oxidative stress is being increasingly implicated as a central etiological factor underlying AD pathology, causing neuronal damage and promoting disease pathology (Ghodake & Suryakar, 2023). The pathologic hallmarks of AD include extracellular A β plaque deposition and intracellular NFTs caused by hyperphosphorylated tau protein. These changes are associated with extensive cellular dysfunction, including oxidative stress, mitochondrial damage, chronic inflammation, and cellular senescence. Collectively, these processes define the complex and interconnected characteristics of AD pathogenesis (Fišar, 2022).

4.2.5.1. Amyloid cascade hypothesis in AD

The amyloid cascade hypothesis proposes that the deposition of A β peptides, which are fragments of APP, initiates the onset of AD and leads to the deposition of amyloid plaques (Chen et al., 2023). The proposed theory predicts that A β deposition initiates a cascade of downstream events like the formation of NFTs, neuronal loss, and vascular injury, which ultimately lead to dementia. Genetic evidence supports this, with indications that mutations in the APP gene located on chromosome 21 have an association with familial AD. Individuals with Down syndrome, having an additional copy of chromosome 21, develop EOAD due to

APP overexpression. Additionally, mutations in the presenilin genes (PSEN1 and PSEN2) greatly influence secretase activity and thereby facilitate A β production (Lyubchenko, 2023).

This hypothesis highlights two features as the main characteristics: the impaired clearance and the pathological production of A β . The enzymatic elements, specifically BACE1, are at the core of A β production, while receptors such as LRP1 and RAGE play a role in the transport activities of A β , along with inflammatory responses in the event of dysfunction. Although it has significantly influenced the development of various therapeutic strategies, the amyloid cascade hypothesis has faced much criticism. These are the concepts that A β might have physiological functions, the lack of good correlation between plaque burden and clinical severity, and the modest success of therapies targeting the reduction of A β levels. Several trials of A β -targeted therapies have failed to demonstrate cognitive improvement. Nonetheless, recent FDA approval of Aducanumab, a monoclonal antibody targeting aggregated A β , has refocused interest on strategies for anti-amyloid therapy (Kepp et al., 2023; Wu et al., 2022).

Inherited AD results from mutations in A β metabolism and clearance processes (Wang, 2023). While the amyloid cascade hypothesis has provided the foundation for AD research, the sequential failure of A β -targeting treatments in clinical trials has created renewed interest in other mechanisms. These include tau protein pathology, neuroinflammation, mitochondrial dysfunction, and synaptic loss. This complex nature of A β toxicity, together with its genetic associations and challenges of effective therapeutic targeting, implies the need for an extended and integrative model for the comprehension and treatment of AD (Andreo-Lopez et al., 2023).

4.2.5.2. Tau and NFTs in AD

Tau is a microtubule-associated protein (MAP) that has the function of neuronal stability, axonal transport regulation, and neurite development. In AD, tau is hyperphosphorylated, which creates NFTs, destabilizes microtubules, and disrupts axonal transport regulation. Hyperphosphorylation is due to increased protein kinase activity and reduced phosphatase activity (Morris & Brady, 2023).

Methylene blue, a tau inhibitor, was not quite effective in enhancing cognitive impairment. Immunotherapy for tau is hopeful, to deplete tau and safeguard nerve cells. NFT growth and maturation in AD brains advance in definite patterns, targeting various regions at different times (Seo et al., 2023). The relationship of amyloid and tau proteins in AD is not clear, although recent studies show that A β may lead to tau phosphorylation. The tau hypothesis proposes that the dramatic actions of tau hyperphosphorylation disrupt axonal transport and trigger neurodegenerative processes. Tau is controlled by the MAPT gene, and disequilibria between

3-repeat (3R) and 4-repeat (4R) tau isoforms have been identified in various tauopathies (Condello et al., 2022). For AD, the problems revolve around the global 3R:4R tau ratio, but 4R tau isoform overexpression is associated with vulnerable brain regions and NFT-containing neurons. Tau's post-translational modifications, particularly phosphorylation, are paramount to its function and causative of tauopathies. Although some phosphorylation is normal, hyperphosphorylation at numerous sites is pathogenic and influences tau binding to both microtubules and AD pathology (Rawat et al., 2022; Seo et al., 2023).

4.2.5.3. The cholinergic hypothesis in AD

The cholinergic hypothesis is that cognitive impairment in AD is caused by acetylcholine neuronal dysfunction. Cholinesterase inhibitors (ChEIs) such as donepezil try to overcome this dysfunction by preventing the breakdown of acetylcholine, with limited cognitive improvement. A variation of this, the cholinergic-vascular hypothesis, is that ChEIs improve cerebral perfusion and that this is the mechanism of their therapeutic effect (Chen et al., 2022).

Following evidence in AD patients, the hypothesis was validated with scopolamine-induced cholinergic blockade causing memory impairment. Despite the limited effect of ChEIs, medial temporal lobe atrophy and the ApoE ϵ 4 allele are predictors of response to treatment. Researchers investigate cholinergic deficiency together with other factors in the pathogenesis of AD. The hypothesis links reduced concentration of acetylcholine to cognitive symptoms, and drugs that inhibit acetylcholine esterase aim to treat AD cognitive symptoms (Blokland, 2022).

Cholinergic deficits cause AD symptoms, particularly in the temporal lobes. NFT formation in AD is staged, supported by MRI and SPECT research showing atrophy and hypoperfusion (Xia et al., 2023). Limbic system atrophy disrupts cholinergic mechanisms and amyloid protein formation, because choline deficiency accounts for AD. Studies show cholinergic deficits that correlate with dementia severity. Elevated cholinergic basal forebrain atrophy rates distinguish AD from normal aging. Glutamate dysregulation, GABAergic remodeling, and deficits in monoaminergic neurotransmission are implicated in AD (Machado et al., 2020).

The cholinergic hypothesis prioritizes the restoration of the cholinergic system in AD treatment based on evidence linking AChE and PS-1 to A β formation and cognitive decline. Pathological changes in the cholinergic system induce inflammation and neuronal apoptosis in AD. In general, the cholinergic hypothesis prioritizes the pathogenicity of cholinergic dysfunction in AD, with evidence supporting cognitive decline and the effectiveness of cholinesterase inhibitors in improving cognitive function (Giacobini et al., 2022).

4.2.5.4. Neuroinflammation in AD

Neuroinflammation, an immune defensive response in the brain, is a component of AD, comprising heterogeneous changes, immune cell activation, and inflammatory mediators involved in neuronal dysfunction. AD immune response is pathological and therapeutic, with potentially protective early-onset inflammation. Chronic immune responses, like those induced by immunogens, require special therapeutic strategies (Yamanaka, 2023).

Neuroinflammation exacerbates AD pathologies that target A β and tau proteins, in which microglia and astrocytes play essential roles in inflammation. Inflammation-related processes in AD are implicated in pathological autophagy, mitophagy, mitochondrial dysfunction, oxidative stress, and senescence (Sobue et al., 2023). Peripheral conditions such as obesity and diabetes enhance inflammation and elevate the risk of AD. Glial cells, mainly microglia and astrocytes, display M1 and M2 phenotypes in neuroinflammation. Targeting neuroinflammation, the NLRP3 inflammasome, and senescence-associated factors is a potential therapeutic strategy for AD. Inflammation, as marked by age-associated immune-metabolic alterations, is responsible for chronic low-grade inflammation in AD (Jain et al., 2023).

The condition has a genetic effect, and presenilin and amyloid precursor proteins are recognized to affect EOAD, and apolipoprotein E (ApoE) polymorphism and immune system genes influence LOAD. Genome-wide genetic studies identify new genes involved in innate immune mechanisms in AD. Understanding the multidimensional interaction between inflammation, genes, and cellular processes is extremely vital to developing effective therapies for AD (Vo & Reynolds, 2023).

4.2.5.5. Oxidative stress in AD

Oxidative stress plays a significant role in the pathogenesis of AD due to its disruption of redox homeostasis and mitochondrial dysfunction. The impairment is responsible for the overproduction of reactive oxygen species (ROS), thus triggering processes like mitophagy, apoptosis, and tau protein phosphorylation. Moreover, oxidative stress is implicated in JNK-mediated apoptotic signaling, neuronal cell cycle disruption, and aberrant modification of tau. Most significantly, amyloid plaque deposition precedes oxidative stress, and the correlation between amyloid deposition and brain glutathione (GSH) is an inverse correlation (Nikolenko et al., 2022; B, 2023).

Mitochondrial dysfunction and trace metal deregulation such as iron, and perturbed glucose metabolism additionally enhance oxidative stress by induction of free radical formation and

amyloid deposition. While modestly low levels of A β are neuroprotective, high A β levels are neurotoxic.

Antioxidant molecules were found to inhibit the aggregation of A β . Meanwhile, oxidative stress plays a role in tau pathology, highlighting the interaction between mitochondrial dysfunction, oxidative damage, and disease pathology.

Changes in oxidative biomolecules and disturbances in transition metal homeostasis also enhance the production of ROS. Copper ions, for instance, facilitate ROS production, resulting in cellular oxidative damage (Cheignon et al., 2023). Together, mitochondrial dysfunction, ROS overproduction, and alteration of oxidative stress biomarkers point to the involvement of oxidative processes in AD. The catalytic activity of metal ions in ROS formation and the resulting oxidative modification of cellular biomolecules highlight the intimate relationship between oxidative stress, metal ion dysregulation, and mitochondrial dysfunction in the complex etiology of AD (Cojocaru et al., 2023).

4.2.5.6. Insulin signaling pathways in AD

Insulin signaling is a fundamental pathway that controls glucose homeostasis, which is triggered by insulin binding to its receptor, autophosphorylation, and stimulation of downstream signaling cascades. Among the most significant aspects of this process is the activation of AKT kinase, which is achieved through phosphorylation at threonine 308 (T308) and serine 473 (S473). This activation results in the translocation of glucose transporters to the cell membrane, thereby facilitating glucose uptake. Insulin signaling dysregulation that exists in type 2 diabetes mellitus (T2DM) has also been more involved in cognitive impairment and increased susceptibility to AD (Ansari et al., 2023).

The hippocampus, a critical area involved in memory processing, shows structural and functional changes in AD, such as A β deposition and tau hyperphosphorylation. Insulin resistance compromises neuroplasticity and facilitates hippocampal dysfunction, inducing neuroinflammation, oxidative stress, and A β pathology. Insulin also plays a regulatory role in A β metabolism and tau phosphorylation. Disrupted insulin signaling in AD is linked to diminished glucose metabolism, compromising neuronal function and viability. Experimental models in rodents demonstrate cognitive deficits and increased tau phosphorylation following insulin pathway impairment (Liu et al., 2022).

Clinical observations also support these results, demonstrating decreased PI3K signaling in patients with Alzheimer's that is associated with increased A β and cognitive decline. Insulin

exerts neuroprotective effects by improving synaptic plasticity, cognition, and neuronal viability. However, in AD, downregulation of insulin receptors occurs, where A β disrupts insulin binding to the receptor and insulin resistance enhances neuropathological processes. The multifaceted interaction between disrupted insulin signaling, A β pathology, and tau pathologies highlights the important role of this pathway in AD pathogenesis and, therefore, its potential as a target for therapy in neurodegenerative disease (Razani et al., 2021).

4.2.5.7. Apolipoprotein E (ApoE) in AD

ApoE4 is a significant risk factor for AD, while ApoE2 is protective. Various combinations of ApoE alleles can influence the onset of AD by 5 to 10 years, and other mutations in the ApoE gene are implicated, especially when there is a high ApoE4/ApoE3 ratio. ApoE is found in every tissue, but the major source is in brain astrocytes. APOE plays pleiotropic functions in lipid redistribution, synaptic plasticity, and cerebrovascular integrity. Yet, ApoE4 can cause neurotoxicity through various cellular mechanisms (Zhang et al., 2023).

The gene has three isoforms (ApoE2, ApoE3, ApoE4) that influence AD pathogenesis by modulating the production of A β , tau neuropathology, and neuroinflammation. ApoE4 carriers have greater A β deposition and plaque that control A β clearance pathways. ApoE participates in inflammation, lipid metabolism, glucose metabolism, mitochondrial function, and blood-brain barrier (BBB) integrity (Sun et al., 2023).

Therapeutically, ApoE lipidation modulation, ApoE/A β targeting, and gene therapy are showing promise in preclinical trials. ApoE genotyping is a potent risk marker for late-onset AD, both for lifetime risk and cognitive decline. It synergizes with modifiable risk factors, suggesting the creation of personalized preventive approaches in ApoE ϵ 4 carriers. Additional research aims at untangling the intricacy of ApoE to enable targeted therapy in AD treatment (Dincer et al., 2022).

4.2.5.8. Autophagy in AD

Autophagy mediated by lysosomes plays a significant role in AD by enhancing the degradation of cellular components and inhibiting the build-up of toxic proteins. It is further categorized into microautophagy, chaperone-mediated autophagy, and macroautophagy, and plays a crucial role in the sustenance of cellular homeostasis via protein and organelle turnover. Various stresses enhance autophagy, which influences cell death, microorganism removal, and tumor suppression (Kapil et al., 2024).

Impaired autophagy accounts for protein accumulation and neurodegeneration. AD, the most common dementia, is a progressive neuronal deterioration with intracellular NFTs and extracellular plaques. AD remains an incurable disorder despite extensive scholarly research, amidst a growing understanding of the indispensable role played by autophagy. Lysosomes, which were discovered by Christian de Duve, are the digestive system of the cell. Proteasomes, which degrade proteins, have been implicated in neurodegenerative diseases. Autophagy, initially characterized by de Duve and further explored by Yoshinori Ohsumi, encompasses intricate cellular processes (Hamano & Endo, 2022).

Common genes between autophagy and AD, including BECN1 and PSEN1, have commonalities with neuroinflammatory pathways. Compounds like rapamycin and curcumin have been directed at modulating autophagy and are being explored in clinical trials for AD treatment. Autophagy's involvement in A β and tau metabolism, synaptic integrity, mitochondrial integrity, and as a therapeutic target constitutes the rationale for its implication in AD pathogenesis and therapy (Vianello et al., 2023).

4.2.6. Risk factors in AD

Risk factors for AD pathology are genetic mutations with at least 24 genes or loci identified, and the ApoE gene e4 allele as the primary risk in non-familial instances. The other risks include Down syndrome, head injury, insulin resistance, and childhood obesity (Allwright et al., 2023).

Clinical AD onset incorporates cardiovascular and cerebrovascular mechanisms whereby juvenile hyperinsulinemia is associated with elevated Ab1-42 and pro-inflammatory cytokines. Risk factors for AD expression in childhood are perinatal conditions, early development, socioeconomic status, environmental enrichment, and cognitive reserve (Loeffler, 2021). Advanced age is a very powerful risk factor for dementia, and the incidence of AD rises with advancing age, so 50% or more of subjects >85 years have AD. The pathological changes of AD are indistinguishable from those that happen in normal aging, so the two are impossible to differentiate from one another.

Genetic predisposition (APP, PSEN1/2, ApoE), lifestyle (alcohol consumption, physical activity, cognitive exercise), disease processes (cancer, cardiovascular illness), and environmental processes (air pollution, metals) all impact AD risk (Eubank et al., 2022). Traumatic brain injury, malnutrition, and calcium imbalance are implicated, with diet diversity, obesity, diabetes, and vascular risk factors also implicated. The immune system, complement proteins, and infections, particularly viruses, are implicated in AD. Psychiatric disorders, such

as depression and persistent psycho-social stress, could contribute to the development of AD (Loeffler, 2021).

4.3. The Relationship Between AD and T2DM

4.3.1. Pathophysiological connections between insulin and AD

The role of insulin in the central nervous system (CNS) extends beyond its traditional role to modulate glucose homeostasis in peripheral tissues. Insulin and insulin receptors are present in various areas of the brain, with exceedingly high concentrations in the hypothalamus, hippocampus, and cerebral cortex. These elements are involved in the modulation of food intake, neurotransmitter release, and synaptic plasticity, which are essential mechanisms of learning and memory (Duarte, 2023; De la Cruz Concepción et al., 2023). The CNS insulin signaling dysfunction has been involved in the pathogenic mechanisms of neurodegenerative disorders, especially AD, and the characterization of AD as "Type 3 diabetes" (Chen et al., 2023). The occurrence of insulin resistance in the CNS associated with AD negatively influences regulation, metabolism, and clearance of amyloid-beta ($A\beta$) and tau proteins, thereby resulting in cognitive decline (Chen et al., 2023).

The regional distribution of insulin receptors within the brain is selective, varying by area and displaying the highest concentrations in the hypothalamus, hippocampus, and cortex. Further, the entry of insulin across the BBB is uneven and exhibits high permeability in areas like the pons, medulla, and hypothalamus (Gralle et al., 2020). For AD, the expression of insulin receptors was noted to be upregulated, and this suggests a possibility of a chronic compensation for insulin resistance. Nevertheless, the advancement in regulatory mechanisms seems not to be enough to thwart the adverse effects accompanying dysregulated insulin signaling. Disruption of insulin pathways plays a pivotal role in neuronal glucose hypometabolism and the ensuing signaling defects, which are paramount to cognitive dysfunction seen in AD (Ezkurdia et al., 2023).

4.3.2. Brain insulin-like growth factor (IGF) resistance

The insulin and insulin-like growth factor (IGF) signaling pathways are central to cognitive processes and the maintenance of neuronal health in the brain. Insulin and IGF binding to their respective receptors activate these pathways, which have a crucial role in the facilitation of neuronal growth, survival, energy metabolism, synaptic plasticity, and neurotransmitter regulation (Fitzgerald et al., 2023). Insulin and IGF pathway changes have the potential to compromise the structural as well as the functional integrity of the CNS, thus promoting the

development and progression of neurodegenerative disorders such as AD (El Idrissi & Alonso, 2022).

In AD, dysregulation of insulin and IGF signaling pathways is closely associated with the formation of pathological features, including the formation of NFTs composed of hyperphosphorylated tau protein and A β deposition. These eventually lead to synaptic dysfunction and cognitive decline (Ma et al., 2022). In addition, disruption of these signaling pathways hinders brain glucose metabolism and energy utilization, promoting neurodegeneration in AD. This metabolic derangement leads to increased oxidative stress, mitochondrial dysfunction, and the stimulation of pro-inflammatory cytokines, all of which contribute to gradual neuronal loss. Elucidation of the complex interactions between insulin/IGF signaling and AD pathogenesis is critical for the development of targeted therapeutic strategies designed to preserve cognitive function and to prevent or slow neurodegeneration in AD and related disorders (Ortiz et al., 2022).

4.3.3. Insulin-degrading enzyme (IDE)

Insulin-degrading enzyme (IDE) plays a significant role in the modulation of insulin balance and A β peptide clearance in the CNS. In addition to its well-known function in insulin degradation, IDE possesses broad substrate specificity and is implicated in peptide degradation that is involved in various metabolic and neurodegenerative disorders (Yilmaz et al., 2023). The primary function of insulin-degrading enzyme (IDE) is its degradation of A β at physiological pH, highlighting its important function in preventing the formation of amyloid deposits, a hallmark pathological feature of AD. Consistent reports of decreased IDE activity in the brains of AD-diagnosed patients, along with elevated A β levels, suggest a causal link between the dysregulation of IDE and AD pathology (Abramov-Harpaz & Miller, 2022).

The bi-directional, dynamic interaction between insulin and IDE also complicates its neurodegenerative role. Insulin at high concentrations can compete with IDE-mediated A β degradation and thereby promote A β accumulation and AD pathology. A β forms, in turn, can suppress A β degradation via IDE, a feedback mechanism augmenting insulin resistance as well as amyloid burden. The bidirectional communication underscores the central role of the enzyme at the intersection of metabolic and neurodegenerative pathways, rendering it an attractive therapeutic target in AD and its affiliated disorders (Kraupner et al., 2022).

Furthermore, genetic investigations have uncovered a harmful role of IDE in the pathogenesis of T2D and the development of AD, thereby showing clear evidence for shared molecular mechanisms in the two disorders. Genetic variations within the IDE gene have been associated

with a higher risk and severity of LOAD and with other clinical expressions of AD pathology. Also, the location of the IDE gene on chromosome 10, close to additional loci associated with susceptibility to AD, renders it an attractive candidate for genetic contribution to disease progression (Martín-Martín et al., 2022).

4.3.4. Amyloidogenesis

The amyloidogenic pathway of AD is complex and entails the misfolding and subsequent deposition of A β peptides into insoluble fibrils, a hallmark pathological feature of the condition. Hereditary variants of AD are typically associated with mutations in the gene encoding APP and therefore act to underscore the fundamental role played by amyloidogenic mechanisms in the initiation and progression of the condition (Andreo-Lopez et al., 2023). The deposition of amyloid plaques, characterized by their extracellular nature and structural composition derived from A β peptides, is a primary causative agent in the formation of synaptic dysfunction, neurodegenerative disorders, and cognitive impairment. The formation of amyloid deposits, either systemically or as localized accumulations, as demonstrated in amyloidosis, represents the extensive pathological activities of amyloidogenesis beyond the CNS (Ma et al., 2022).

Current therapeutic strategies for the reversal of amyloidogenesis in AD involve a broad range of mechanisms. These are inhibition of A β aggregation, correction of familial AD gene mutations, correction of protein misfolding, modulation of NFT formation, and direct elimination of amyloid aggregates (Kumar & Roy, 2023). Induction of autophagic pathways, improvement of cerebral blood flow, and neuronal protection against A β toxicity are some other targets for therapy. Clarification of the molecular pathways that regulate amyloidogenesis and its downstream effects is critical for the development of novel approaches to preventing Alzheimer's disease pathology. Current studies in this area hold promises for the development of more efficacious, disease-modifying therapies for Alzheimer's disease and its complications (Sharari et al., 2023).

4.3.5. cGMP dysregulation in type 2 diabetes and Alzheimer's disease

Reduced cGMP levels make a dramatic difference in the cellular signaling mechanisms and the functions related to them, with implications as wide-ranging as other pathologies such as T2D and AD (Jehle & Garaschuk, 2022). In T2D, reduced cGMP levels shown in models such as Zucker diabetic fatty (ZDF) rats may be involved as being linked with cerebral insulin resistance and which may be affected by mitochondrial dysfunctions and oxidative stress. Analogously, aging insulin resistance associated with low levels of cGMP is in accord with chronic neuroinflammation and augmented BBB permeability, which are the early features of

AD pathology. These observations highlight the common pathophysiological processes of T2D and AD and point to cGMP dysregulation in their pathogenesis (Vargas-Soria et al., 2022).

In addition, the role of phosphodiesterases (PDEs) in cGMP breakdown introduces yet another facet to this multifaceted interaction. Enhanced PDE activity, as seen in the adipose tissue of individuals with T2D, could also play a role in cardiovascular comorbidities of the disease (Friebe et al., 2023). As cGMP is one of the major players in various cellular processes, including smooth muscle relaxation, vascular inflammation, synaptic transmission, and neuronal plasticity, its reduction in level can be of catastrophic consequence. Reduced levels of cGMP have been involved in cognitive impairment and amyloidogenesis in AD, and thus, its involvement has been emphasized in neurodegenerative processes (Arancio et al., 2022).

4.4. Treatment of Alzheimer's Disease

4.4.1. Pharmacological treatment of AD

Pharmacologic treatment of AD, with ACh inhibitors like donepezil, rivastigmine, and galantamine, opposes neurotransmitter deficiency and improves non-cognitive symptoms. Effectiveness may be limited by multifactorial pathology in dementia. Deficiency of several neurotransmitters, like 5-hydroxytryptamine and glutamate, needs to be identified for optimal benefit. Memantine, in moderate to severe illness, has tolerability benefits and acts on non-cognitive symptoms (Manoj Sarjerao & Pallavi Tukaram, 2022).

4.4.1.1. Cholinesterase inhibitors

Cholinesterase inhibitors serve as the cornerstone of AD treatment through the augmentation of cholinergic neurotransmission. This is based on evidence that reveals the decline in cortical activity of the ChAT enzyme and the degeneration of cholinergic neurons in areas of the brain affected by the disease (Walczak-Nowicka & Herbet, 2021). The inhibitors, including donepezil, rivastigmine, and galantamine, do this by increasing the levels of acetylcholine, which are crucial for processes of attention and learning. Further, experimental data indicate a potential link between cholinergic deficiency and amyloid plaque and NFT formation in Alzheimer's disease, which implicates cholinesterase inhibitors in modulating Ab aggregation and fibril formation (Ure et al., 2023). Donepezil, rivastigmine, and galantamine are the most widely used cholinesterase inhibitors for the management of mild to moderate AD.

Donepezil, a derivative of N-benzylpiperidine, was first approved by the FDA in 1996 as a reversible and selective inhibitor of AChE to treat AD. Donepezil enhances cholinergic

transmission by inhibiting the degradation of acetylcholine, reclaiming cognitive and activities of daily living function in mild to moderate AD (Pooladgar et al., 2022).

Pharmacokinetically, donepezil is quickly absorbed when administered orally, with peak plasma levels in 3–4 hours. It is extensively protein-bound and has a very long half-life of 70–80 hours, which is conducive to once-daily dosing. Metabolized primarily through CYP3A4, it requires caution in severe hepatic impairment, but it is otherwise well tolerated in the elderly patient with mild organ impairment. Donepezil has a very good safety profile, and common side effects include nausea, diarrhea, dizziness, and muscle cramps. Severe adverse reactions like bradycardia are rare. Its preferential selectivity over butyrylcholinesterase for AChE is the reason behind enhanced tolerability (Odenigbo et al., 2024; Zheng et al., 2024).

Clinical trials have shown that donepezil improves cognitive function over three weeks and maintains its effects with long-term administration. While it does not change the course of the disease, it considerably delays cognitive decline and reduces the caregiver's burden, particularly when it is part of a well-integrated management strategy (Warren & Moustafa, 2022).

Rivastigmine is an FDA-approved pseudo-irreversible dual AChE and BChE inhibitor for the treatment of mild to moderate AD and Parkinson's disease dementia in 2000. Rivastigmine is a selective G1 isoform inhibitor of AChE, most expressed in AD, and enhances cholinergic neurotransmission through the elevation of brain acetylcholine levels, particularly in the cortex and hippocampus (Gupta et al., 2021).

Pharmacokinetically, rivastigmine is rapidly absorbed ($T_{max} \approx 1$ hour) with low plasma protein binding and extensive CNS distribution. Hydrolysis by cholinesterases and not by hepatic CYP450 enzymes is the mechanism of action, minimizing drug-drug interaction risk. It has a brief plasma half-life (~1 hour) compared to a prolonged pharmacodynamic effect, supported by sustained metabolite availability (e.g., NAP 226-90 in CSF for up to 15 hours). The transdermal patch provides increased patient compliance and tolerability by delivering a maximum amount of drug release with fewer gastrointestinal side effects (Spencer & Noble, 1998).

Side effects are frequent and consist of dose-related nausea, vomiting, diarrhea, and weight loss with oral preparations. The patch reduces these risks significantly. The infrequent but severe adverse effects are bradycardia and gastrointestinal bleeding. Rivastigmine's metabolism avoids hepatic enzymes, making it perfect for polypharmacy, particularly in elderly or hepatic/renal-impaired patients (Pagliuca et al., 2023).

Its efficacy in improving cognition, daily function, and delaying institutionalization has been proven through clinical trials. Combination treatment with memantine was synergistic in moderate to severe AD. Long-term efficacy may be modest, but rivastigmine is an effective first-line therapy, especially in individuals who are intolerant or unresponsive to other cholinesterase inhibitors (Nguyen et al., 2020).

Galantamine, a selective acetylcholinesterase inhibitor, was approved in 2001 by the FDA for treating mild to moderate AD. It was initially derived from plants like daffodils and snowdrops, but it is currently synthesized in nearly all cases (Atrahimovich et al., 2021). Galantamine has preferential inhibition of acetylcholinesterase over butyrylcholinesterase and is a unique allosteric modulator of nicotinic acetylcholine receptors with a particular affinity for the $\alpha 7$ subtype, thus facilitating cholinergic transmission and indirectly regulating glutamatergic, serotonergic, dopaminergic, and GABAergic systems (Philipova et al., 2020).

Pharmacokinetically, galantamine is rapidly absorbed orally, with the time to peak plasma concentration at 0.5–2 hours for immediate release and ~4.4 hours for extended release. Food delays absorption but not bioavailability. It is low plasma protein bound (~18%) and undergoes extensive hepatic metabolism via CYP2D6 and CYP3A4. About 75% of the dose is hepatically inactivated, and the drug and metabolites are largely eliminated renally. It has an elimination half-life of 5.5 to 8 hours. Moderate to severe renal or hepatic impairment with reduced clearance also needs to be cautious (Aanandhi, 2022).

Side effects are gastrointestinal (nausea, vomiting, anorexia), dizziness, and weight loss in larger doses. These are generally mild and disappear with dose titration or ongoing use. Serious but rare side effects are QT prolongation, bradycardia, and skin reactions such as bullous pemphigoid. Gastroenteric intolerance is lessened with the utilization of extended-release preparation (Katib et al., 2024).

Galantamine improves memory, attention, and functional ability in early to moderate phases of AD. It is initiated at 8 mg/day and titrated to 16–24 mg/day based on tolerance and response. Its dual action of AChE inhibition and nicotinic receptor modulation is responsible for its cognitive and neuropsychiatric symptom amelioration, with a number of Phase III trials supporting the evidence. Clinical trials demonstrate that galantamine improves cognitive function and activities of daily living performance at 3–6 months, and longer trials show efficacy up to 12 months. This dose increase brings additional benefits, particularly for cognition and behavior. Galantamine does not halt AD but postpones deterioration and institutionalization, especially when it started early in the disease process (Lim et al., 2024).

Further, variability in pharmacokinetic profiles, tolerability, and adverse effects among drugs within this class necessitates an individualized approach to treatment strategy and monitoring. While the therapeutic benefit of cholinesterase inhibitors is largely of moderate extent, the medications can induce cognitive improvement and disease slowing and may potentially benefit from the attenuation of neuropsychiatric and behavioral symptoms. However, the use of maintenance therapy should be evaluated in consideration of potential adverse effects, expense, and total benefit to the patient. Discontinuation of treatment is indicated in the absence of clinical improvement or when a further deterioration in cognition or function occurs. Long-term effectiveness and the theoretical basis for the utilization of cholinesterase inhibitors remain contentious, necessitating additional high-quality comparison trials to determine their role in the treatment of Alzheimer's disease (Mecocci et al., 2020).

4.4.1.2. Memantine

Memantine is a non-competitive antagonist of the NMDA receptor and a keystone treatment of moderate to severe Alzheimer's disease. Memantine improves behavior, daily functioning, and cognitive function without causing psychosis. When added to a stable dose of donepezil, memantine reduces agitation, apathy, and language function, possibly synergistically interacting with other treatments (Tang et al. 2023; Terry et al. 2021). Although not presently advocated in NICE guidelines, memantine has a useful niche in the management of behavior and psychological symptoms of dementia amidst the background of safety concerns with antipsychotics in this population.

Glutamate excitotoxicity, by overactivation of NMDA receptors and intracellular calcium influx, accounts for neuronal demise in Alzheimer's disease. Memantine selectively inhibits such noxious currents without influencing normal synaptic transmission, thereby decreasing excitotoxicity and enhancing cognitive and functional outcomes, particularly when coupled with cholinesterase inhibitors (Yu et al. 2023). It is generally well tolerated, excreted unchanged by the kidneys, and has a simple titration schedule that begins with 5 mg per day and goes up to 10 mg twice a day (Althobaiti 2020). Initial studies indicate benefits even at the initial phases of Alzheimer's disease, substantiating its usage after stabilization of cholinesterase inhibitor therapy.

By modulating synaptic glutamatergic transmission to enhance normal NMDA receptor function and block abnormal excessive overactivation, memantine improves global function, behavioral symptoms, and cognition along the continuum of the disease. The effectiveness of

its action and favorable safety profile highlight memantine's value as part of a comprehensive Alzheimer's disease treatment strategy (Li et al. 2021).

4.4.2. New approaches for the treatment of AD

Alzheimer's disease has many symptoms, including loss of memory, cognitive impairment, and changes in behavior. This has made research into treatments imperative. Aducanumab is the first FDA-approved medication that targets amyloid plaques. It can slow down the decline in thinking skills. Other medications, such as lecanemab, are undergoing clinical trials (Jiang & Xiao, 2022). Scientists are also exploring non-pharmacological treatments coupled with off-label medication use of drugs like NSAIDs and lithium for managing symptoms. Despite these advances, drug development in AD continues to be beset by high failure rates, as evidenced by recent failures with BACE1 inhibitors and solanezumab, which point to the need for several molecular targets and earlier intervention to curtail the predicted worldwide rise in dementia incidence (Brent 2023).

4.4.2.1. Aducanumab

Aducanumab was approved by the United States Food and Drug Administration (FDA) on June 7, 2021, and represents the first treatment for Alzheimer's disease that is targeted to decrease cerebral amyloid-beta ($A\beta$) deposition, one of the characteristic pathologies of the disorder. Biogen developed this medication in collaboration with Eisai and is a selective human IgG1 monoclonal antibody to certain amino acid residues of $A\beta$ (Egl3, Phe4, Arg5, His6) to facilitate the clearance of amyloid plaques (Wu et al., 2023; Tu, 2024).

In spite of its innovative mechanism of modifying disease progression, aducanumab's therapeutic efficacy, particularly its ability to improve cognition, remains contentious due to conflicting trial results and modest functional benefits (Thussu et al., 2024).

Pharmacokinetically, aducanumab reaches maximum plasma concentration ($\sim 182.7 \mu\text{g/mL}$) at three hours after intravenous infusion with a half-life of ~ 25 days. Steady state is reached after ~ 16 weeks of multiple dosing. The medication is administered at a fixed dose of 10 mg/kg monthly after titration, irrespective of sex, age, or body weight. Despite dose-dependent amyloid removal, modest clinical effect necessitates continued MRI monitoring because of the threat of amyloid-related imaging abnormalities (ARIA). The limited genetic influence, e.g., from ApoE, acts on exposure, and the therapy does not reverse the basic pathology (Beshir et al., 2022).

Aducanumab as a pharmacologic treatment reflects a departure from the conventional symptomatic treatments, such as cholinesterase inhibitors and NMDA antagonists, memantine, to disease-modifying treatments intended to change the basic pathology of Alzheimer's disease. Its approval and use as a therapy, though, have been restricted by extremely high expenses, safety issues, and modest therapeutic benefits (Ashique et al., 2024).

Clinical trials were mixed. Phase Ib (PRIME) determined dose-related amyloid reduction. Phase II was aimed at ARIA safety. In Phase III, the EMERGE trial showed 22% less clinical worsening in the high-dose arm compared to placebo, but the ENGAGE trial failed to demonstrate a significant benefit, continuing controversy surrounding the overall value of the drug in practice (Heidebrink & Paulson, 2024).

4.4.2.2. Lecanemab

Lecanemab (BAN2401) is a humanized IgG1 monoclonal antibody under development by Eisai and Biogen that is designed to target toxic beta-amyloid protofibrils specifically to decrease their accumulation and slow cognitive decline in those with early Alzheimer's disease. The antibody was granted fast-track accelerated approval by the FDA in January 2023 and regular approval in July 2023, pending positive clinical trial results and a robust safety and efficacy profile. This is the second anti-amyloid treatment to be approved by the FDA and is also under regulatory review in the European Union, Japan, and China (Zhu & Abossi, 2024; Jin & Noble, 2024).

Pharmacokinetically, intravenous Lecanemab 10 mg/kg biweekly has achieved steady-state at six weeks and 1.4-fold accumulation. A subcutaneous formulation is in development but is found to exhibit lower peak plasma concentrations and lower bioavailability, estimated at around 49.7% relative to intravenous infusion. Its pharmacokinetics can be influenced by a number of factors that include weight, sex, serum albumin level, presence of anti-drug antibodies, and ethnicity, especially in Japanese populations, while age does not seem to have any influence (Willis et al., 2023).

Lecanemab has selective binding to aggregated soluble and insoluble A β , that is, protofibrils, reducing amyloid plaques, reducing plasma p-tau181, and increasing the A β 42/40 ratio. This is opposed to drugs that target tau. Clinical trials indicated that biweekly IV dosing significantly reduces amyloid burden and slows cognitive decline, as measured by ADCOMS, CDR-SB, and ADAS-Cog14, with a good safety profile (Hayato et al., 2022).

Infusion reactions, headache, and amyloid-related imaging abnormalities (ARIA-E and ARIA-H) are common adverse events; these adverse effects, however, are mild and self-limiting in nature in most instances. In the Phase III CLARITY AD study, a 27% decrease in the rate of cognitive worsening progression was noted with no significant increase in severe adverse effects compared to the placebo group. Approximately 12.6% of the patients experienced mild ARIA development, which is typically resolved in months (Reisa et al., 2024).

Lecanemab has shown consistent efficacy in preclinical, Phase I, IIb, and III studies with good brain penetration and great clearance of plaques. While overall well tolerated, long-term trials are needed to determine its sustainability and long-term tolerability in Alzheimer's disease (Smith et al., 2025).

4.4.2.3. Donanemab

Donanemab (Kisunla) is a monoclonal antibody of the human IgG1 type for early Alzheimer's disease in individuals with mild cognitive impairment or mild dementia. Donanemab acts against a truncated, pyroglutamate-modified beta-amyloid (N3pG) form, an important component of cerebral plaques, and promotes clearance via microglial phagocytosis. This treatment can minimize neuroinflammation without interfering with neuronal function (Shukla & Misra, 2023).

Donanemab, after being approved in the United States in July 2024 and subsequently in other regions, is given via intravenous infusion once every four weeks. The medication features a low volume of distribution and is eliminated by the reticuloendothelial system and proteolytic degradation with a therapeutic half-life of 20-30 days. It is not metabolized by cytochrome P450 enzymes, thus having reduced drug-drug interaction potential (Song et al., 2024).

Donanemab lowers amyloid plaque burden and slows cognitive deterioration by as much as 38.6%, with the greatest impact in low to moderate tau pathology patients. Progression of the disease was delayed in clinical trials by 7.5 months, and 47% of those taking the drug had no progression after one year in comparison to 29% on placebo (Sims et al., 2023).

Medication is well tolerated in patients. Side effects include infusion procedure-related reactions, headache, and amyloid-related imaging abnormalities (ARIA) that are mostly mild and temporary. ARIA's severe forms were seen in about 1.6% of the subjects in the studies (Mintun et al., 2023).

Briefly, Donanemab is a disease-modifying treatment approach with a novel mechanism that targets amyloid-beta variants and adds to the expanding pharmacological arsenal for treating early Alzheimer's disease.

4.4.3. Other pharmacotherapeutic strategies

4.4.3.1. Antioxidants

Antioxidants such as vitamins E and C play a crucial role in mitigating oxidative stress linked to Alzheimer's disease through the neutralization of free radicals and maintenance of neuronal integrity. Vitamin E, specifically the alpha tocopherol form, is protective against lipid peroxidation, neuronal cell death, and inflammation; evidence indicates that supplementation can slow disease progression while also reducing amyloid-beta deposition and the plaque burden in the brain (Knight et al., 2023). Estrogen is an antioxidant, and through this property, it can reduce neuronal inflammation and amyloid-beta (A β) formation in postmenopausal women. Caffeine intake within normal limits has been linked with decreased A β levels, less plaque deposition, and reduced cognitive deterioration, while its excessive consumption can produce adverse effects (Veurink et al. 2020). Curcumin from turmeric demonstrates both anti-inflammatory and antioxidant properties by inhibiting A β aggregation and oxidative stress. Several compounds, including ubiquinone, alpha-lipoic acid, acetyl-L-carnitine, creatine, docosahexaenoic acid, Ginkgo biloba extracts, and Huperzine A, have yielded promising but mixed results in preclinical and clinical trials, highlighting the necessity for additional rigorous trials to determine their effectiveness (Varshney & Siddique, 2024).

Dietary habits high in foods containing antioxidant properties also provide neuroprotection. The MIND diet, high in berries, nuts, seeds, and leafy green vegetables, has been linked to a lower risk of Alzheimer's disease and slowing of cognitive deterioration. Clinicians should advise patients to utilize a balanced diet high in natural antioxidants to keep the brain healthy and possibly slow the course of the disease. Although the US Preventive Services Task Force has not provided official recommendations on antioxidant supplementation for the prevention of Alzheimer's, supplementation with more antioxidant-containing foods is consistent with overall dietary recommendations for healthy aging and could have added benefits in decreasing the risk of dementia (Pritam et al. 2022).

4.4.3.2. Anti-amyloid pharmacostategies

Anti-amyloid treatment in Alzheimer's disease is aimed at reducing the synthesis of A β , its deposition, and its toxicity. Gamma secretase inhibitors have surprisingly worsened cognitive

impairment in clinical trials, and beta secretase inhibitors are being tried in early disease. Activation of protein kinase C, which enhances the activity of alpha secretase, has been found to decrease the levels of A β 42 in animal models experimentally (Sharari et al. 2023). Such strategies are designed to inhibit amyloidogenesis through the specific modulation of secretase activity without disrupting vital biological pathways such as notch signaling (Imbimbo et al. 2023).

Besides secretase inhibition, another treatment includes the utilization of small-molecule inhibitors of A β aggregation or proteasome-activating compounds. Tramiprosate, clioquinol, and colostrinin are additional medications that also function as amyloid assembly disruptors. What's more, rosiglitazone, an enzyme modulator, promoted cognitive improvement within the preclinical models of Alzheimer's disease by inhibiting A β formation (Moore et al. 2022).

Immunotherapeutic treatments such as active vaccination with A β peptides and passive administration of monoclonal antibodies such as solanezumab and bapineuzumab attempt to remove amyloid plaques that are already formed. Although early clinical trials demonstrated heterogeneity of results, novel antibodies and optimized dosing regimens are being tested for effectiveness to preserve cognitive function (De Lorenzi et al. 2023). Additional studies addressing combination therapies with novel anti-amyloid medications are required to advance the focus of Alzheimer's disease treatment.

4.4.3.3. ApoE as a therapeutic target in AD

Anti-ApoE therapies offer hopeful opportunities for the management of Alzheimer's disease through targeting ApoE4, a key genetic risk factor. Strategies to reduce ApoE4 levels, such as antisense oligonucleotides (ASOs) and small interfering RNAs, have shown improved efficacy and bioavailability (Zhai et al. 2023). Small molecules and therapies with CNS specificity targeting the IDOL/PCSK9-LDLR-ApoE pathway also have therapeutic promise. Immunotherapies and gene therapy targeting distinct ApoE isoforms demonstrate benefit in preclinical models. Together, these interventions seek to alleviate pathology, enhance cognition, and modulate neuroinflammation, offering hope for disease-modifying treatments soon (Sun et al. 2023).

Regulation of the ApoE level and modification of its properties are of therapeutic priority. The upregulation of ApoE by agonists of nuclear receptors reduces the A β load and improves cognitive function in animal models of Alzheimer's disease. Structural modification of ApoE and regulation of the ApoE lipidation status are other potential intervention points. Though

dosing optimization and safety are yet concerns, such strategies present great potential for impending Alzheimer's disease therapies (Valencia et al. 2023).

ApoE-target therapies also engage in indirect mechanisms like the restoration of BBB integrity and the regulation of ApoE receptors like LDLR and LRP1. These are mechanisms that increase A β clearance and reduce neuroinflammation. Additionally, apoE4-specific therapies that disrupt the ApoE-A β interaction are able to modulate peptide aggregation and deposition to halt disease progression (Raulin et al. 2022).

The current AD pipeline comprises symptomatic therapies to treat agitation, insomnia, and other behavioral disturbances and disease-modifying therapies against misfolded proteins such as A β and tau. Immunotherapy, neuroprotective agents, and regenerative approaches are designed to improve cognitive function and synaptic plasticity, while anti-inflammatory approaches are aimed at microglial modulation and risk genes such as TREM2. Lessons from failed trials in the past point to the need to expand therapeutic targets and treat in prodromal or even preclinical phases (Decourt et al. 2022). Recent studies are investigating new areas including the modulation of neurotransmitter systems; serotonin and histamine receptor antagonistic agents have shown promising efficacy, in addition to drug repositioning strategies to reduce developmental costs and preventive trials to delay the onset of dementia in high-risk population groups (Cunliffe et al. 2022; Lopes et al. 2021).

4.5. T2DM Drugs in AD Treatment

4.5.1. Effects of T2DM drugs for the treatment of AD

Pharmacologic drugs used for diabetes, i.e., metformin, sulfonylureas, and GLP-1 receptor agonists, are under investigation for their pharmacologic potential for therapeutic applications in AD. These drugs are potentially therapeutic against the pathology of AD, such as protein misfolding, cerebral edema, and neuronal apoptosis. They also reduce high blood glucose levels, increase insulin sensitivity, and preserve cell structure, thus preventing inflammation and oxidative stress in AD (Maková et al., 2023). Metformin suppresses hepatic glucose production, increases glucose uptake, and allows brain cells to function by reducing amyloid-beta and preventing tau protein modification in Alzheimer's models. Thiazolidinediones promote brain cell growth, decrease brain inflammation, and reverse abnormalities caused by tau. DPP-4 inhibitors sitagliptin, raise brain GLP-1 levels and have been shown to improve cognition (Harding et al., 2023).

Metformin and sulfonylureas have also been shown to be linked with a 24 percent and 15 percent lowered risk of dementia, respectively, in observational studies (Tian et al., 2023). While initial results and certain clinical reports, i.e., recovery of memory and mood following intranasal insulin therapy, are promising, more clinical trials must be undertaken to confirm these findings as well as define the long-term efficacy and safety of anti-diabetic drugs for the therapy of neurodegenerative disorders. Considering the heterogeneity of the treatment regimens and the possible damage to brain tissue, it remains challenging to establish the absolute contribution of such medications to the risk of dementia (Mantik et al., 2023).

4.5.2. Intranasal insulin

Intranasal insulin is being developed as a novel treatment for AD and related cognitive dysfunction. Since this route of delivery introduces insulin directly into the brain without crossing the BBB, it is not an invasive and selective treatment with reduced systemic side effects. Preclinical and clinical trials all pointed to enhancement of memory, attention, and synaptic plasticity, and cognition was reported in a dose-dependent fashion (Taléns-Visconti et al., 2023). Compared to peripheral insulin administration, intranasal administration is safer concerning hypoglycemia and enhances CNS function. It is also found to reduce phosphorylated tau, increase neurogenesis, and confer overall neuroprotection. All these actions are highly relevant to AD pathology, where tau accumulation and neuronal damage are typical (Nene & Santoni, 2023).

Experimental evidence also shows that intranasal insulin modulates microglial activation and astrogliosis, improves insulin signaling pathways in the brain, and inhibits amyloid-beta aggregation. Clinical trial participants who received intranasal insulin also exhibited better memory, mood, and cognition without any noticeable alteration in peripheral insulin or glucose levels, indicating its safety and tolerability (Wu et al., 2023; Ahmad, 2022). While promising, additional studies are needed to optimize dosing regimens, genotype-specific responses, and long-term safety. However, intranasal insulin has enormous potential as a symptomatic and disease-modifying therapy for Alzheimer's disease.

4.5.3. Metformin

Metformin, a biguanide medication used in the treatment of type II diabetes, has exhibited neuromodulatory actions above and beyond mere glycemic regulation. By acting through the activation of molecular cascades such as AMPK/BDNF1/p70S6K2, metformin has been demonstrated to improve learning, memory, and alleviate anxiety-like behavior in diabetic animal models (Bora & James, 2023). Further, its neuroprotective effects include the

modulation of autophagy, diminution of neuroinflammation, and inhibition of neuronal loss—processes of interest to the pathophysiology of neurodegenerative diseases such as AD.

Recent clinical evidence indicates that administering metformin could reverse cognitive impairment and enhance memory performance in diabetic and non-diabetic patients (Harding et al., 2023). Preclinical research continually demonstrates that metformin might be aiding in the treatment of Alzheimer's disease through inhibiting the accumulation of amyloid-beta ($A\beta$), improving spatial memory, and inducing microglial autophagy. These promising results do not adequately translate to human patients. While some epidemiological studies have shown an association between metformin therapy and reduced risk of developing Alzheimer's disease, other studies have found that prolonged exposure to the drug increases the risk of cognitive impairment, particularly vascular dementia and Alzheimer's disease, in diabetic patients (Wang et al., 2023).

The contradictory findings underscore the intricate nature of metformin's effect in the context of AD. While preclinical models demonstrate their ability to reduce levels of $A\beta$ and tau phosphorylation, inhibit inflammatory responses, and reduce oxidative stress, the variability of clinical findings necessitates more focused investigations. Clinical trials in the future must stratify participants by their glycemic control, baseline cognitive status, and diabetes history to refine the therapeutic application of metformin in AD (Zheng et al., 2023).

4.5.4. Sulfonylureas and glinides

Sulfonylureas (SUs), a class of anti-diabetic medications that facilitate the secretion of insulin by acting on ATP-sensitive potassium (K_{ATP}) channels of pancreatic β -cells, have been found to have neuroprotective properties in preclinical models of AD. Empirical findings point to SUs, particularly glibenclamide and glipizide, being able to reduce hippocampal amyloid-beta ($A\beta$) accumulation, inhibit neuronal apoptosis, and enhance synaptic plasticity. It is noteworthy that the combination of metformin with sulfonylureas (SUs) co-administration has provided a 35% reduction in the risk of developing dementia, whereas long-term SUs monotherapy has displayed no impact on the progression of Alzheimer's disease, as confirmed through clinical evidence (Wu et al., 2023). Although SUs are inexpensive, they have a high potential for inducing hypoglycemia and thereby inducing cognitive impairment, especially in the elderly. SUs' therapeutic potential in modifying AD pathology is less ideal compared to metformin. Preclinical data do indicate, however, that SUs can suppress $A\beta$ deposition and inflammation.

Besides their use in the control of blood glucose levels, sulfonylureas like glibenclamide and gliclazide have also been shown to be effective in diabetes research through the inhibition of

inflammation and opposition of oxidative stress. The drugs oppose oxidative stress by boosting the body's general antioxidant defense mechanisms that are geared towards limiting lipid damage and lowering the amount of toxic agents in cerebral tissue. Sulfonylureas produce their pharmacological effects by binding to the sulfonylurea receptor (SUR1) subunit of K_{ATP} channels, which are also expressed in the CNS. The channels, composed of Kir pore-forming subunits and SUR regulatory subunits, play a role in cognitive function by regulating neural excitability and calcium homeostasis. Besides, glibenclamide demonstrated the potential to enhance depression- and anxiety-like behaviors as well as being involved in enhancing cognitive function in a number of animal models. These actions seem to be mediated by blockade of the SUR1-gated TRPM4 channel, an action associated with neuroinflammation and apoptosis of cells. By inhibiting this interaction, glibenclamide can potentially limit CNS damage and neuronal degeneration (Maqoud et al., 2022). Although initial evidence from preclinical studies is encouraging, additional clinical trials are required to determine the therapeutic potential of sulfonylureas for the management of AD, particularly in terms of dosage, safety, and criteria for patient selection.

4.5.5. Thiazolidinediones

Thiazolidinediones (TZDs), such as pioglitazone and rosiglitazone, are a group of anti-diabetic medications that are agonists of the peroxisome proliferator-activated receptor gamma (PPAR- γ). These drugs increase insulin sensitivity, decrease hepatic gluconeogenesis, and may exhibit neuroprotection against neurodegenerative disorders, including AD. Preclinical research has shown that TZDs might reduce neuroinflammation and oxidative stress and reduce the amounts of toxic proteins that are normally found in Alzheimer's disease (SLong et al., 2021).

Research has demonstrated that pioglitazone exerts profound neuroprotection through inhibition of the hyperactive activity of cyclin-dependent kinase 5 (Cdk5) and the levels of the P35 protein. These are important in controlling tau hyperphosphorylation in models of Alzheimer's disease. It was also found to improve cerebrovascular function, restore cholinergic function, reduce oxidative stress, and normalize cerebellar protein expression, which ultimately results in the improvement of insulin sensitivity and cognitive functions (Hwang et al., 2021).

Rosiglitazone has also demonstrated equivalent advantages in reducing amyloid-beta (A β) pathology and enhancing cognitive function through PPAR- γ -mediated pathways. However, its limited ability to cross the BBB, in addition to its association with increased cardiovascular risks, has greatly hindered its clinical application, rendering pioglitazone an improved prospect for investigation and therapy in the realm of AD (Saunders et al., 2021).

Clinical trials assessing the impact of pioglitazone on AD patients with or without T2DM have indicated a positive effect on cognitive function and cerebral blood flow. Despite the promising nature of these findings, the differences between clinical and preclinical findings, attributable to variations in trial duration, design, and populations, indicate that further research is needed (Alhowail et al., 2022).

Apart from glucose regulation, TZDs also appear to have actions on the central processes of AD, like inhibition of tau phosphorylation, lowering of A β burden, activation of neuronal survival, and promotion of synaptic plasticity. Population data are accumulating to suggest that TZDs are more effective than metformin in reducing dementia incidence, at least in Asian populations (Feng, 2023; Lin et al., 2023). These findings enhance the argument for examination of the potential of pioglitazone as an Alzheimer's disease modifier, with the caveat of robust, large-scale clinical evidence.

4.5.6. Glucagon like peptide-1 (GLP-1)

GLP-1 receptor analogues and agonists, such as liraglutide and lixisenatide, were originally developed for the therapy of type 2 diabetes, in which they promote insulin secretion, suppress the release of glucagon, and enhance insulin sensitivity. In addition to their metabolic effects, these medications have shown neuroprotective properties in preclinical models, with reductions in oxidative stress, neuroinflammation, and amyloid plaque deposition, and with enhancement of synaptic plasticity. By stimulating cyclic AMP (cAMP) signaling in the brain, GLP-1 receptor agonists promote neurogenesis and synaptogenesis and thus enhance memory impairments and prevent neurodegeneration in AD models (García-Casares et al., 2023).

Liraglutide was shown in clinical trials to have therapeutic effects, which include improving glucose metabolism and slowing down the impairment in cognitive processes in AD patients. Additionally, GLP-1 receptor agonists have demonstrated potential for the treatment of Parkinson's disease (PD) through modulation of the PI3K-Akt signaling pathway, inhibition of GSK-3 β , decrease in toxic protein aggregation, regeneration of axonal structure, and decrease in neuroinflammation. Preclinical studies with medications like exenatide, semaglutide, and NLY01 have established their neuroprotective actions in PD models, whereas initial clinical trials indicate that exenatide can improve motor and cognitive function (Chen et al., 2023).

GLP-1 receptor agonists such as liraglutide and exenatide are being repurposed to treat dementia based on evidence of their capacity to prevent memory loss, neuronal loss, and loss of synaptic plasticity in the hippocampus. Mechanistically, they suppress β -secretase and γ -secretase activity to limit amyloid-beta (A β) formation, stimulate the PI3K/MAPK pathways to

increase A β clearance, and improve mitochondrial function to facilitate neuronal survival. However, even with these encouraging results, the therapeutic efficacy of GLP-1 receptor agonists in dementia is yet to be determined, and current clinical trials will more likely determine their potential to alter the trajectory of AD and enhance cognitive outcomes (Bi et al., 2023).

4.5.7. Amylin analogue

Amylin, a hormone co-secreted with insulin upon glucose intake, has an important function in the regulation of blood glucose and gastric emptying. Amylin dysfunction, as is commonly associated with obesity and pre-diabetic insulin resistance, leads to its oligomerization and has been involved in the pathogenesis of T2DM. Pramlintide, the FDA-approved analogue of amylin utilized in T2DM, is active against hyperamylinemia and inhibits the release of toxic amylin aggregates (Casadesus et al., 2022).

CNS amylin also has similar pathological features to A β , including the formation of toxic oligomers and fibrils. Preliminary research showed that pramlintide decreases oxidative stress and neuroinflammation and enhances memory function in AD models in mice. While clinical data in humans are still limited, the evidence indicates that pramlintide is an extremely promising therapeutic agent for diabetic brain damage and AD (Corrigan et al., 2023).

Amylin's function in AD is still multifaceted, and some have argued that upregulation of amylin receptors reverses A β toxicity. Pramlintide treatment has been linked to enhanced cognitive performance, decreased amyloid plaque burden, and decreased oxidative stress in models of AD. Pramlintide safety in non-diabetic AD patients has also been determined in initial clinical trials and has shown that therapeutic potential may lie in its activity in the modulation of lipid metabolism, opening up novel therapeutic avenues (Casadesus et al., 2022).

4.6. The Role of Dipeptidyl Peptidase-4 Inhibitors (DPP-4Is) in AD

4.6.1. Regulation of DPP-4I's in the AD brain

Dipeptidyl peptidase-4 (DPP-4) inhibitors are an important component of AD by enhancing the neuronal insulin resistance and mitochondrial dysfunctions via augmentation of GLP-1 signaling. DPP-4 inhibitors have been found to enhance memory behaviors and spatial learning in AD mouse models. DPP-4 inhibitors offer neuroprotection against A β cytotoxicity, attenuation of GSK3 β activation, and suppression of tau hyperphosphorylation, thus facilitating memory retention. Additionally, they elevate GLP-1 in the brain, minimize nitrosative stress and neuroinflammation, and decrease A β deposition (Siddiqui et al., 2023).

Combination therapy using DPP-4 inhibitors and memantine has been indicated to exert additive effects in reducing APP levels and phosphorylated tau, thereby enhancing cognitive deficits and influencing major determinants of AD. Such determinants involve incretin signaling, amyloid-beta ($A\beta$) deposition, tau phosphorylation, and neuroinflammatory responses. Besides that, DPP-4 inhibitors also show time-dependent improvement of memory performance, preservation of synaptic proteins, amelioration of mitochondrial function in the brain, and inhibition of neuronal cell death in AD models (Hu et al., 2022).

Additionally, research on rats on a high-fat diet (HFD) highlights the connection between brain mitochondrial dysfunction, characterized as increased production of ROS, mitochondria depolarization, and swelling, and defective ATP synthesis, leading to cognitive impairment (Kelty et al., 2023).

4.6.2. Effects of DPP-4Is for the treatment of AD

DPP-4 inhibitors, including gliptins like saxagliptin, linagliptin, vildagliptin, and sitagliptin, raise GLP-1 levels to manage T2DM with minimal effect on body weight or gastric emptying. These drugs can be used either as monotherapies or in combination with other antidiabetic medications, with a lower risk of hypoglycemia and potential benefits for the management of AD. Empirical research has determined that gliptins, specifically sitagliptin and vildagliptin, are shown to enhance cognitive function, mitigate mitochondrial injury, and delay $A\beta$ accumulation in AD models. The results of the effect of sitagliptin on the development of AD are conflicting, but linagliptin was discovered to have the potential to reverse cognitive function deficits as well as suppress neuroinflammation in preclinical studies (Du et al., 2022).

DPP-4 inhibition not only enhances insulin secretion but also appears to have a beneficial impact on cognitive function in diabetics and lowers the risk of developing Parkinson's disease. Linagliptin, for example, is neuroprotective through the reduction of cytotoxicity, improvement of cognitive outcomes, and inhibition of tau hyperphosphorylation in AD mouse models. DPP-4 inhibitors have been successful in maintaining cognitive function in elderly patients with mild cognitive impairment and AD. In addition, GLP-1 analogs like liraglutide have shown potential in inhibiting memory loss, neurodegeneration, and amyloid plaque formation in AD. Clinical studies show that liraglutide may have the capacity to reduce the rate of decline in cerebral glucose metabolism among AD patients (Mackin & Currie, 2022).

Preclinical models also suggest that DPP-4 inhibitors like saxagliptin and vildagliptin stabilize GLP-1, leading to reduced $A\beta$ deposition, tau phosphorylation, and inflammatory markers. Sitagliptin possesses antioxidative and anti-apoptotic activities on neuroinflammation,

enhances the antioxidative response, suppresses apoptosis, and activates synaptic proteins with O-glycosylation to permit cognitive enhancement. Saxagliptin and linagliptin, however, exert beneficial effects on learning and memory functions through the modulation of GLP-1/GLP-1 receptor (GLP-1R) signaling, incretin levels, and insulin signaling pathways. Furthermore, vildagliptin has revealed distinctive mechanisms that augment A β and tau clearance, thereby leading to cognitive enhancement in AD models (Mehan et al., 2022).

In general, DPP-4 inhibitors are mostly focused on oxidative stress and inflammation via utilization of the GLP-1/GLP-1R pathway, thereby improving cognitive functions by influencing the production and clearance of pathogenic proteins. The clinical trials of the cognitive impacts of sitagliptin and vildagliptin are, nonetheless, scant, emphasizing the need for more studies on the therapeutic outcomes of DPP-4 inhibitors in AD, potentially in combination with additional anti-AD medications (Gilbert & Pratley, 2020).

4.6.3. Vildagliptin

Vildagliptin, a DPP-4 inhibitor, increases the levels of GLP-1 and release of insulin and suppresses the hepatic glucose production simultaneously, and this accounts for some of the enhanced glycemic control in streptozotocin-diabetic rats. It also exhibits anti-hyperglycemic, anti-inflammatory, and antioxidant effects; reverses cognitive deficits; lowers serum glucose and lipid profile; and improves brain BDNF and SOD levels. Its working mechanism is by increasing the levels of GLP-1, enhancing the function of pancreatic β -cells, reducing insulin resistance, and restoring the mass of pancreatic β -cells (Zhang et al., 2021).

GLP-1 in diabetes enhances insulin secretion through potassium and calcium channel action to an increased cyclic adenosine monophosphate (cAMP) level that continues to augment neurotransmitter release. Vildagliptin is a DPP-4 inhibitor that elevates endogenous levels of GLP-1, which has the potential to decrease A β deposition in the brain. Long-term DPP-4 inhibition has been found to have protective effects on AD pathology, making it a promising therapeutic strategy (Hu et al., 2022).

Vildagliptin, an incretin-type drug for the treatment of type 2 diabetes, enhances insulin secretion, improves insulin sensitivity, and normalizes blood glucose in diabetic rat models. It also improves peripheral insulin resistance, prevents cognitive decline, decreases oxidative stress in brain tissue, and enhances brain insulin sensitivity and mitochondrial function in obese, insulin-resistant rats. Nonetheless, vildagliptin does not prevent weight gain and completely reverse synaptic impairment in the brain (Titov et al., 2022).

In insulin-resistant obese rats, vildagliptin treatment has been found to improve mitochondrial function in the brain and allow signaling of insulin, thus precluding cognitive dysfunction, with the same effects as being exerted by dapagliflozin. Vildagliptin's neuroprotective effect, involving regulation of A β and tau clearance through the Klotho protein and Akt signaling pathway, also positions it in a viable therapeutic role in the management of AD (Zhang et al., 2021).

4.7. The Role of Sodium-Glucose Cotransporter Inhibitors (SGLTIs) in AD

4.7.1. Regulation of SGLTIs in the AD brain

Sodium-Glucose Cotransporter-2 (SGLT2) inhibitors improve cognitive function through the reduction of oxidative stress, enhancement of insulin sensitivity, and promotion of memory consolidation and neuronal survival. SGLT2 inhibitors also elevate mammalian target of rapamycin (mTOR) signaling in the brain, thereby improving the clearance of excess glucose resulting from insulin resistance. SGLT2 inhibitors are beneficial in preventing cognitive impairment, maintaining synaptic plasticity in the hippocampus, and minimizing neuronal cell apoptosis. Also, by reducing A β accumulation and alleviating tau-related pathological brain atrophy, SGLT2 inhibitors help in preserving neurological well-being (Lato et al., 2023).

In T2DM animal models of diabetes, mTOR inhibition induced by SGLT2 inhibitors has been shown to exert beneficial effects on tau-associated cerebral atrophy, potentially recuperating metabolic homeostasis and averting the danger of metabolic and mitochondrial malfunction in Alzheimer's disease (Piątkowska-Chmiel et al., 2023). SGLT2 inhibitors also reduce oxidative stress in the brain, enhance mitochondrial function, and inhibit neuronal apoptosis, all of which cumulatively result in a lower risk of AD. SGLT2 inhibitors also enhance synaptic plasticity in the hippocampus and inhibit A β deposition at the same time. Through the restoration of mTOR signaling and the maintenance of glucose homeostasis, SGLT2 inhibitors may delay the development of Alzheimer's disease pathology (Yaribeygi et al., 2024). SGLT2 inhibitor therapy also increases circulating levels of ketone bodies that are able to modulate major pathological pathways, enhance cognitive function, and reduce oxidative stress in diabetic mouse models (Lu et al., 2022).

4.7.2. Effects of SGLT2 inhibitors for the treatment of AD

SGLT2Is decrease the plasma glucose level through the increased excretion of glucose in the urine, lowering the fasting and postprandial glucose levels and the HbA1c level. Beyond glycemia, SGLT2i inhibitors show marvelous benefits with fewer hospitalizations for heart

failure, cardiovascular deaths, and kidney benefits in patients with T2DM. The effectiveness of the SGLT2i drug dapagliflozin is also applicable to the state of heart failure with reduced ejection fraction (HFrEF) regardless of the presence of T2DM (Jasleen et al., 2023).

These inhibitors impact cardiac, renal, and cerebral health by restoring circadian mTOR activity and normalizing anabolic/catabolic cycling. They induce a metabolic shift toward catabolic over anabolic processes, relying on fatty acid oxidation (FAO) and ketogenesis for brain fueling. SGLT2 also regulates AMPK activity and inhibits mTOR transiently, providing glucose-lowering independent effects and possibly lowering Alzheimer's disease pathology (Podestà et al., 2023).

SGLT2 inhibitors such as Sotagliflozin have action on both the SGLT1 and SGLT2 co-receptors and, therefore, influence glucose regulation in the CNS. Although Canagliflozin has the highest SGLT1 inhibitory activity, Empagliflozin and Ertugliflozin are more selective for SGLT2. The degree of this selectivity differs with various inhibitors and affects both their neuroprotective action and their interaction with other systems (Rizzo et al., 2022). There is evidence of a link between T2DM and heart failure due to chronic glucotoxicity that results in myocardial structural and functional defects. SGLT2 inhibitors are beneficial in keeping glucose in check and lowering cardiovascular risk. A 1% increase in HbA1c is associated with a 25% higher risk for cardiovascular events or death in patients with T2DM (Santulli et al., 2023).

In neurodegenerative diseases of Alzheimer's and Parkinson's, SGLT2 inhibitors have shown promise in blocking brain shrinkage, amyloid pathology, and improving memory. Pharmacological agents have neuroprotective effects by inducing autophagy, mitigating oxidative stress, and enhancing insulin sensitivity. Clinical evidence indicates decreased incidence of dementia among SGLT2 inhibitor-treated patients, with dapagliflozin showing the most significant decrease. Long-term therapy with SGLT2 inhibitors has been reported to be associated with better cognitive function, particularly in elderly diabetic patients, with a promise of benefit for reducing dementia risk (Osmanović Barilar et al., 2023).

4.7.3. Empagliflozin

SGLT2 inhibitors like empagliflozin (EMP) have an insulin-independent action and therefore augment the function of β -pancreatic cells and preserve insulin levels. Evidence suggests that EMP administration in diabetic mouse models decreases tau hyperphosphorylation, an essential characteristic of AD, through improvement of insulin resistance and restoration of the equilibrium between tau kinases and phosphatases. It has also been demonstrated that EMP

lowers amyloid pathology through improvement of oxidative stress, inhibition of inflammation, and improvement in the integrity of the BBB (Choi et al., 2023).

Studies report a decreased risk of dementia in older adults with T2D treated with EMP, pointing to its role in regulating AD pathology. EMP's effect on cognition and memory improvement in diabetic mice is due to its ability to decrease markers of oxidative stress in the brain and bring about neuroprotection. Chronic EMP treatment of mouse models of AD and T2D has led to reductions in brain pathology, neuronal loss, microglial inflammation, and senile plaque load, and also to learning and memory enhancement. These findings suggest EMP's potential in addressing the brain pathology of both AD and T2D (Alhowail et al., 2022).

In murine models of mixed AD and T2D, treatment with EMP markedly ameliorated cognitive impairments and memory function, as supported by assays like the new object discrimination test. Such an effect is because EMP has been shown to improve memory and neuronal survival, while decreasing oxidative stress markers and improving proteins crucial for cognitive processes (Hierro-Bujalance et al., 2020).

4.8. The Role of a Combination SGLT2Is and DPP-4Is in AD

Medications like SGLT2 inhibitors and DPP4 inhibitors can improve cognitive function in metabolic dementia by reducing blood glucose levels, improving insulin resistance, and elevating cerebral glucose metabolism. SGLT2 inhibitors reduce blood glucose levels independently and improve insulin resistance, and they may ameliorate dementia by reducing inflammation and normalizing the use of energy (Wu et al., 2022). DPP4 inhibitors increase active GLP-1 levels, improving glucose regulation and potentially preventing dementia by modulating apoptosis, neuroinflammation, and synaptic plasticity. Both classes of drugs have shown neuroprotection in Alzheimer's disease models, although additional research is needed to fully determine their therapeutic functions (Sim et al., 2021). In animal models, SGLT2 and DPP4 inhibitors improve metabolic profiles, insulin resistance, and glucose tolerance, indicating advantages in late type 2 diabetes.

They enhance insulin signaling in the brain, inhibiting tau phosphorylation and A β deposition, the characteristic pathophysiological features of Alzheimer's disease. The medications also stimulate MasR and modulate the RAS system, promoting cognitive function and reducing brain oxidative stress. They also reduce hepatic lipid accumulation, inflammation, and promote glucose homeostasis, which can improve brain function in metabolic dysfunction-related dementia (Osman et al., 2023).

Clinical research demonstrates that DPP4 inhibitors can reduce the deterioration of memory and decrease the deposition of amyloid in individuals with type 2 diabetes. In contrast, SGLT2 inhibitors illustrate a decreased incidence of dementia alongside indications of augmented protective measures versus Parkinson's disease. The two drug classes have demonstrated potential to decrease dementia risk and enhance cognitive functions, and this can allude to the possibility of preventative and therapeutic uses regarding cognitive dysfunctions of type 2 diabetes (Au et al., 2022).



5. MATERIALS AND METHODS

5.1. Chemical Substances

Glucose, streptozotocin (STZ), and vildagliptin (Galvus) were obtained from Novartis Pharmaceuticals Corporation (AG, Basel, Switzerland). Empagliflozin (Jardiance) was obtained from Boehringer Ingelheim (Ingelheim am Rhein, Rhineland-Palatinate, Germany).

Insulin (Humulin®) was acquired from Eli Lilly (Indianapolis, IN, USA).

5.2. Devices

This thesis study was conducted at the Department of Pharmacology, Faculty of Pharmacy, Marmara University, and the Marmara University Research and Application Center for Experimental Animals. The devices used:

- Open Field Test Cage
- MAY Passive Avoidance Device
- Morris Water Tank

5.3. Experimental Animals

In this thesis study, 40 Sprague-Dawley rats weighing between 250-350 g were used. Ethical approval for the study was obtained from the Local Ethics Committee for Animal Experiments at Marmara University (86.2017.mar) before the study was conducted. The rats used in the study were obtained from the Marmara University Experimental Animals Research and Application Center, and the animal experiments for the thesis were carried out at this center. Throughout the experiment, the animals were kept in an environment with a 12-hour light/12-hour dark cycle, a temperature of 22 ± 2 °C, and 60% humidity. They were provided with continuous access to food and water.

5.4. Experimental Groups

In this thesis study, Sprague-Dawley rats were divided into 2 groups:

I. Control group rats (n=8): The animals were fed with standard rat chow for 8 weeks, and at the end of the 4th week, they were injected with physiological saline (1 ml/kg, i.p.) to mimic streptozotocin (STZ).

II. Model group rats (n=32): The animals were fed with a HFD containing 57% energy for 8 weeks, and at the end of the 4th week, they were given a low dose (25 mg/kg, i.p.) of STZ (Ozturk, 2016). One day after the STZ administration, the rats' blood glucose was measured with a glucometer; rats with blood glucose levels above 200 mg/dl were considered diabetic and divided into 4 groups, and the drug administrations were given via intragastric gavage. Distilled water was given intragastric gavage to the animals in control and T2DM-induced AD groups.

II.1. T2DM-induced AD group (n=8)

II.2. T2DM-induced AD + Empagliflozin group (n=8): 10 mg/kg/day (Sim et al., 2023)

II.3. T2DM-induced AD + Vildagliptin group (n=8): 4 mg/kg/day (Powell et al., 2014)

II.4. T2DM-induced AD+ Empagliflozin + Vildagliptin group (n=8): (5 mg/kg/day + 2 mg/kg/day)

5.5. Evaluation of The Diabetic Status of Experimental Animals

5.5.1. Measurement of body weights of experimental animals

The body weights of the rats were measured at the beginning of the study (week 0) and at the start of each subsequent week (weeks 1, 2, 3, 4, 5, 6, 7, and 8).

5.5.2. Weekly blood glucose measurement, and oral glucose tolerance test (OGTT) administration

Weekly blood glucose levels were measured using a glucometer on blood samples taken from the tail vein of rats at any time of day, without prior fasting, half an hour after the treatments were administered. This procedure was repeated once a week between weeks 4 and 8 of the study.

The oral glucose tolerance test (OGTT) was conducted to evaluate the rats' ability to regulate glucose metabolism and the changes in blood glucose levels following glucose administration. In the 7th week of the experiment, treatments were administered after a 12-hour fasting period, and 30 minutes later, a 2 g/kg glucose solution was given Intragastric gavage. Blood glucose levels were measured with a glucometer from blood collected via the tail vein at the following time points: 0, 30, 60, 90, and 120 minutes after glucose injection.

5.6. Evaluation of Cognitive Functions of Experimental Animals

5.6.1. Open field test (OFT)

The open field test (OFT) was conducted to assess spontaneous locomotor activity and anxiety-like responses in experimental animals. The apparatus consisted of a square Plexiglass arena ($50 \times 50 \times 40$ cm) with an open top and a black, non-reflective floor unfamiliar to the animals. The floor was divided into 25 equal squares (10×10 cm each), creating two zones: the peripheral zone (the 16 squares along the walls) and the central zone (the 9 squares in the middle of the arena). Each rat was individually placed in the center of the arena and allowed to explore freely for 5 minutes. Between each trial, the arena was thoroughly cleaned with ethanol to eliminate residual olfactory cues. All sessions were recorded using an overhead video camera (Tieu, 2020).

Locomotor and behavioral performance were evaluated based on five distinct parameters. The distance traveled was quantified by counting the number of squares crossed, providing an index of general locomotor activity (Paul et al., 2007). The number of rearing events were recorded as instances where the rat stood upright on its hind limbs, reflecting exploratory motivation (J, 2017). The frequency of grooming was measured by counting the number of grooming bouts (licking, scratching, or face-washing), indicating emotional or stress-related behavior (J, 2017). The latency to first crossing into the central zone referred to the time elapsed from the start of the test until the animal first entered the central zone, with a shorter latency interpreted as reduced anxiety. Lastly, the total time spent in the central zone was recorded, where increased time spent in this area indicated lower anxiety levels. The combination of these five measures provided a comprehensive assessment of both motor performance and emotional reactivity under novel environmental conditions (Gellért & Varga, 2016).

5.6.2. Novel object recognition test (NORT)

The Novel Object Recognition (NOR) test is a widely used behavioral assay designed to assess recognition memory in rodents, capitalizing on their natural exploratory preference for novel objects. The test was conducted in an open-field arena made of black Plexiglass ($50 \times 50 \times 40$ cm) under semi-dark conditions. To prevent olfactory bias, both the arena and objects were thoroughly cleaned with ethanol after each trial.

The NOR protocol consists of two consecutive days: a habituation phase and a testing phase. On Habituation Day (Day 1), all animals ($n = 4$) from the same cage were placed into the open-field arena without any objects and allowed to explore freely for 60 minutes to minimize novelty-induced anxiety. This procedure was repeated for all groups to ensure familiarization

with the testing environment. On Testing Day (Day 2), the testing phase involved two separate sessions (T1 and T2), each lasting 3 minutes and separated by a 60-minute intersession interval.

T1 (Familiarization Phase): Two identical objects were placed symmetrically in the arena. Each animal was individually placed in the center and allowed to explore the objects for 3 minutes. Afterward, the animal was returned to its home cage.

T2 (Recognition Phase): Following a 60-minute retention interval, one of the familiar objects was replaced with a novel object. The animal was again placed into the arena and allowed to explore both the familiar and novel objects for 3 minutes.

Both sessions were recorded using a computer-assisted video camera. During T2, the animal's interactions with the objects (e.g., sniffing, licking, touching, climbing) were scored. The discrimination index, reflecting the ratio of time spent exploring the novel object relative to the total exploration time, was calculated as a measure of recognition memory performance. This test provides valuable insights into both learning and memory capacities, allowing researchers to assess cognitive function in animal models (Vila, 2023).

5.6.3. Passive avoidance test (PA)

The Passive Avoidance (PA) test is a behavioral assay designed to assess both short-term and long-term memory retention through a fear-motivated stimulus. It leverages the natural tendency of rodents to seek dark environments when placed in a novel setting. The apparatus for this test consists of two adjacent compartments (each measuring $20 \times 25 \times 30$ cm) separated by a partition wall with a small transition door. One compartment is illuminated, while the other is kept dark with opaque walls. Both compartments feature grid floors made of stainless-steel rods, capable of delivering an electric foot shock.

The procedure is performed over two consecutive days. On Day 1 (acquisition phase), each rat is individually placed in the illuminated compartment. When the animal spontaneously enters the dark compartment, it receives a mild foot shock (40 V, 0.5 A, 5 seconds) through the grid floor. After the shock, the rat is immediately removed and returned to its home cage.

The retention test is conducted 24 hours later. In this session, the rat is again placed into the illuminated compartment, and the latency to enter the dark compartment is recorded. No electric shock is administered during this phase. If the rat does not enter the dark compartment within 300 seconds, the trial is terminated, and a maximum latency of 300 seconds is recorded. The test is used as an indicator of memory retention, with longer latencies reflecting better memory

performance. All sessions are carried out under controlled environmental conditions to ensure consistency and reproducibility (Lorente Picón, 2023).

5.6.4. Morris water maze test (MWM)

The Morris Water Maze (MWM) test is a widely recognized and rigorously validated behavioral assay used to evaluate spatial learning and memory in rodents. In this study, the MWM apparatus consisted of a circular stainless-steel pool with a diameter of 160 cm and a height of 40 cm. The pool was conceptually divided into four equal quadrants using four fixed external cues positioned around the testing area to assist in spatial navigation. A hidden escape platform, measuring $10 \times 10 \times 10$ cm, was placed in one predetermined quadrant and remained in the same location throughout the acquisition phase. To eliminate visual cue interference, the platform was constructed from a material matching the color of the pool and submerged approximately 1.5 cm below the water surface.

During the acquisition trials, each rat was gently placed into the water, facing the wall, from various starting points located in quadrants that did not contain the platform. Each trial had a maximum duration of 75 seconds, during which the animal was required to locate the hidden platform. If the rat failed to find the platform within the allotted time, it was gently guided to the platform and allowed to remain there for 20 seconds to reinforce learning. Training trials were conducted over five consecutive days, with each animal undergoing daily sessions consisting of several trials, separated by approximately 10-minute intervals to minimize fatigue and stress. The primary outcome measured during the acquisition phase was the escape latency, defined as the time taken by the animal to reach the hidden platform.

A probe trial was conducted 24 hours after the final acquisition session to assess memory retention. During the probe trial, the platform was removed from the pool, and each rat was allowed to swim freely for 60 seconds. Parameters evaluated during the probe trial included the time taken to reach the former platform location (target quadrant) and the percentage of time spent within the target quadrant compared to other quadrants. These measures served as indicators of spatial memory consolidation and retrieval. All behavioral activities were recorded using a video tracking system, allowing for subsequent quantitative analysis (Nunez, 2008)

5.7. Termination of the Experiment

After the 8-week treatment period, the experiment was terminated at the beginning of the 9th week by decapitating the rats under deep anesthesia induced with anhydrous diethyl ether.

Relevant tissues were then collected for the biochemical and histological analyses described below.

5.8. Statistical Analyses

Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). All data are presented as mean $\pm$ standard error of the mean (SEM). A two-way analysis of variance (ANOVA) was used for the OGTT and Morris water maze tests, while one-way ANOVA was applied for the other tests. Tukey's post hoc test was used for group comparisons. A p-value of <0.05 was considered statistically significant.



6. RESULTS

6.1. Evaluation of the Diabetic Status of Experimental Animals

6.1.1. Evaluation of weight measurements

The body weights of the experimental animals were measured before the start of the study (at the beginning of week 0) and at the beginning of weeks 1, 2, 3, 4, 5, 6, 7, and 8 to document weekly weight changes. Weekly weight measurements of the animals are presented in Figure (1). Differences in body weight among the animal groups were observed throughout the experimental period.

In the evaluation of the experimental groups (excluding the control group), a gradual increase in body weight was observed during the first four weeks. Following the administration of a low dose of streptozotocin (STZ) in week 4, a significant decrease in body weight was recorded in most groups.

The T2DM-induced AD group showed a gradual increase in weight up to week 4, followed by a less pronounced increase. The empagliflozin group showed a marked weight reduction starting from week 5. The vildagliptin group exhibited relative weight stability with a slight tendency toward reduction in the later weeks. The group treated with both empagliflozin and vildagliptin showed an initial increase in weight during the early weeks, followed by a period of relative stability. However, none of these changes were statistically significant.

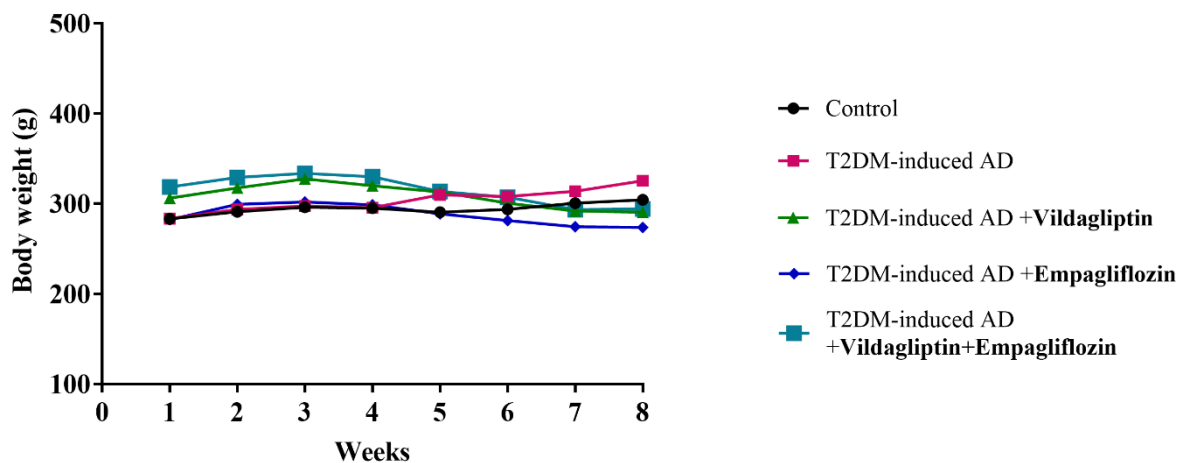


Figure 1. Animal body weights (g) by week

Data are presented as mean $\pm$ SEM, $n = 8$ per group. Body weight was measured at baseline (week 0) and at the beginning of each week through week 8. Data were analyzed by two-way ANOVA (group $\times$ time) followed by Tukey's post hoc test. No significant differences were observed between groups (ns, $p > 0.05$).

6.1.2. Evaluation of blood glucose measurements

Blood glucose levels (mg/dL) were measured weekly in the experimental animals from week 1 to week 8 using a blood glucose meter. Blood samples were collected from the tail vein 30 minutes after treatment administration to assess the changes induced by the different pharmacological treatments, as presented in Figure 2.

The control group maintained stable blood glucose levels throughout the experimental period, with no significant changes observed. In contrast, the T2DM-induced AD exhibited a progressive and marked increase in glucose levels beginning in week 5, peaking at week 7, and remaining elevated through week 8. This increase was statistically significant compared to the control group ($p < 0.001$).

All treated groups showed relatively lower glucose levels compared to the T2DM-induced AD, with statistically significant differences as follows:

In the T2DM-induced AD + Vildagliptin group, a moderate increase in glucose levels was observed after week 4, with significant differences compared to the T2DM-induced AD group during weeks 5 to 8 ($p < 0.05$, $p < 0.01$, and $p < 0.001$, depending on the week).

In the T2DM-induced AD+ Empagliflozin group, the increase was less pronounced, with significant reductions compared to the T2DM-induced AD over the same period ($p < 0.01$ and $p < 0.001$). The T2DM-induced AD + Vildagliptin + Empagliflozin group showed the lowest glucose levels among the treated groups, with highly significant differences compared to the T2DM-induced AD group during weeks 5 to 8 ($p < 0.001$).

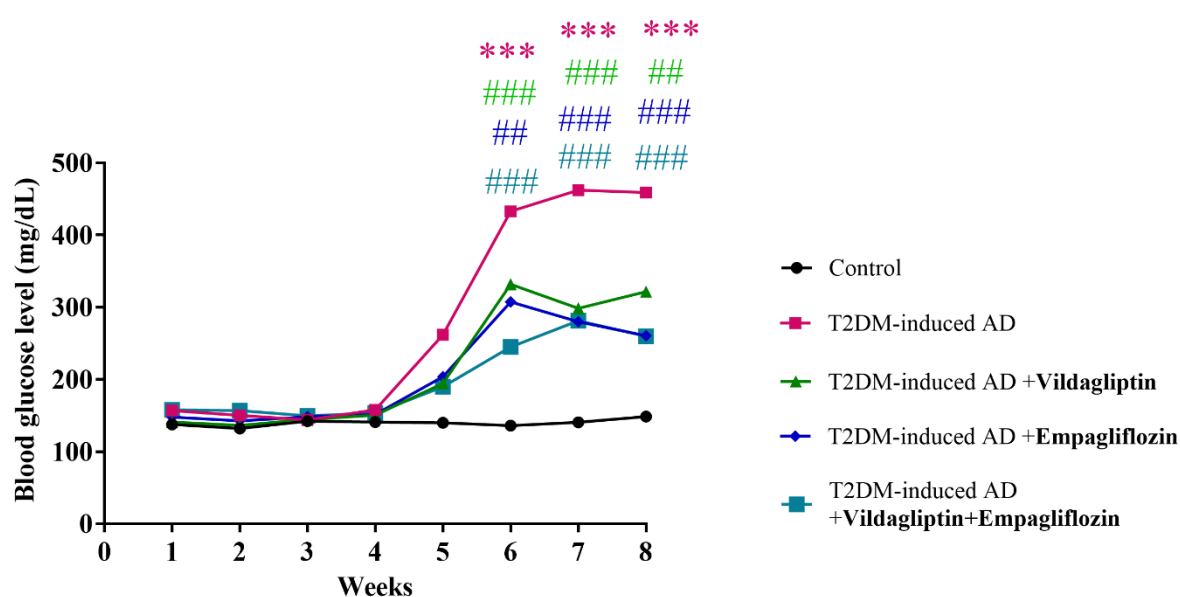


Figure 2. Blood glucose (mg/dl) by week (mean $\pm$ SEM, n = 8 per group)

Statistical analyses were performed using two-way ANOVA and Tukey as post-test using GraphPad Prism software. *** $p < 0.001$ versus Control; $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ versus T2DM-induced AD.

6.1.3. Evaluation of oral glucose tolerance test measurements

OGTT was performed in the seventh week to evaluate glucose regulation and glycemic response in rats. Following a 12-hour fasting period, the animals received their respective oral treatments 60 minutes before glucose administration. Subsequently, glucose (2 g/kg) was delivered intragastrically. Blood glucose levels were measured at 0 (baseline), 30, 60, 90, and 120 minutes using a glucometer, with samples collected from the tail vein.

As presented in Figure 3, the T2DM-induced AD group exhibited a marked and sustained elevation in blood glucose levels compared to the control group ($p < 0.001$) at 60 minutes and remaining elevated through 120 minutes. In contrast, the control group maintained consistently lower and stable glucose levels throughout the test period.

The vildagliptin and empagliflozin group demonstrated a modest decrease in glucose levels; however, these changes were not statistically significant at most time points.

The combination group (empagliflozin + vildagliptin) exhibited a pronounced glucose peak at 30 minutes, which was significantly higher than the T2DM-induced AD (## $p < 0.01$) and numerically greater than all other groups. This was followed by a rapid and considerable decline in glucose levels at subsequent time points.

These findings reflect distinct glycemic response profiles among the experimental groups during the oral glucose challenge.

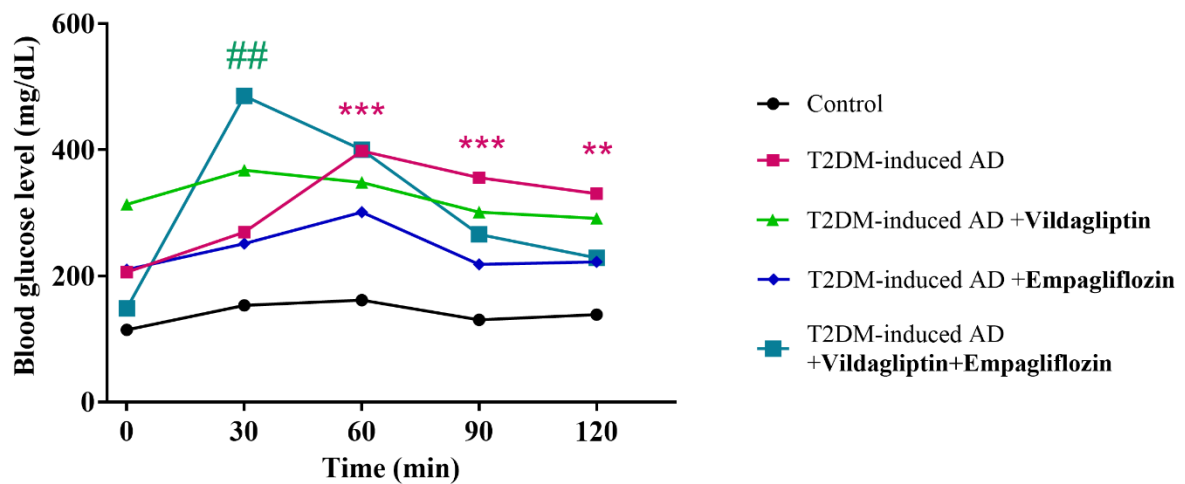


Figure 3. Blood glucose (mg/dl) over time according to oral glucose tolerance test

Data are presented as mean $\pm$ SEM (n = 8 per group). Statistical analyses were performed using two-way ANOVA and Tukey as post-test using GraphPad Prism software. *** p < 0.001 versus Control; ## p < 0.01 versus T2DM-induced AD.

6.2. Evaluation of Cognitive Status of Experimental Animals

Behavioral testing of the experimental animals was carried out in the 7th and 8th weeks of the 8-week experimental protocol.

6.2.1. Evaluation of open field test results

The OFT was employed to evaluate locomotor activity, exploratory behavior, and anxiety-like responses in experimental rats. Behavioral parameters assessed included rearing and grooming frequency, the number of line crossings, latency to enter the center zone, and the duration spent in the center area.

As illustrated in Figure 4, no statistically significant differences were observed in the number of rearing events across experimental groups. Both the T2DM-induced AD and treatment groups displayed rearing frequencies comparable to the control group, suggesting no major alterations in vertical exploratory behavior. Similarly, grooming behavior, shown in Figure 5, did not differ significantly among the groups. This indicates that none of the treatments had a measurable effect on grooming frequency, a parameter often linked to stress or anxiety-related responses.

Locomotor activity, as indicated by the number of squares crossed (Figure 6), was also comparable among all groups. No significant differences were detected, implying that neither

the disease model nor the administered treatments had a notable impact on general motor function during the test period.

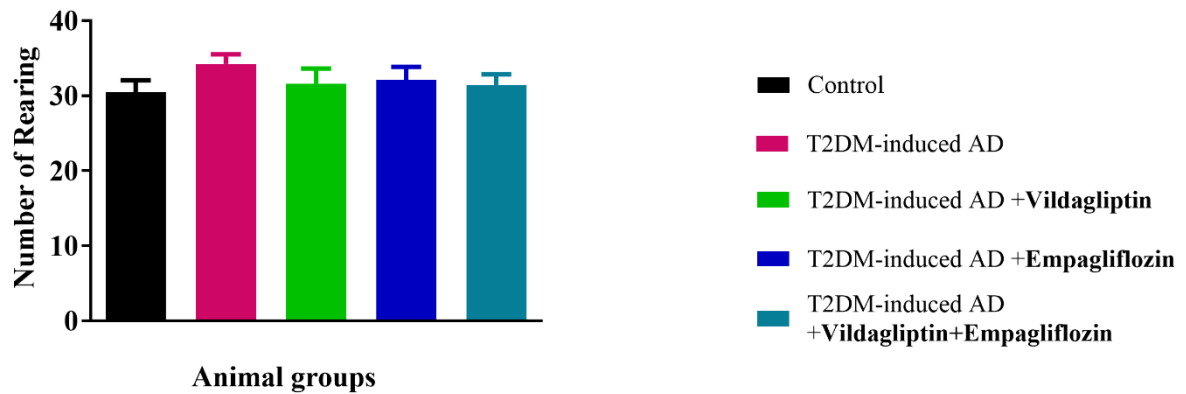


Figure 4. Number of rearing in the open field test. Data are presented as mean ± SEM (n = 8 per group)

Statistical analyses were performed using one-way ANOVA and Tukey as post-test using GraphPad Prism software. No significant differences were observed among groups (ns, $p > 0.05$).

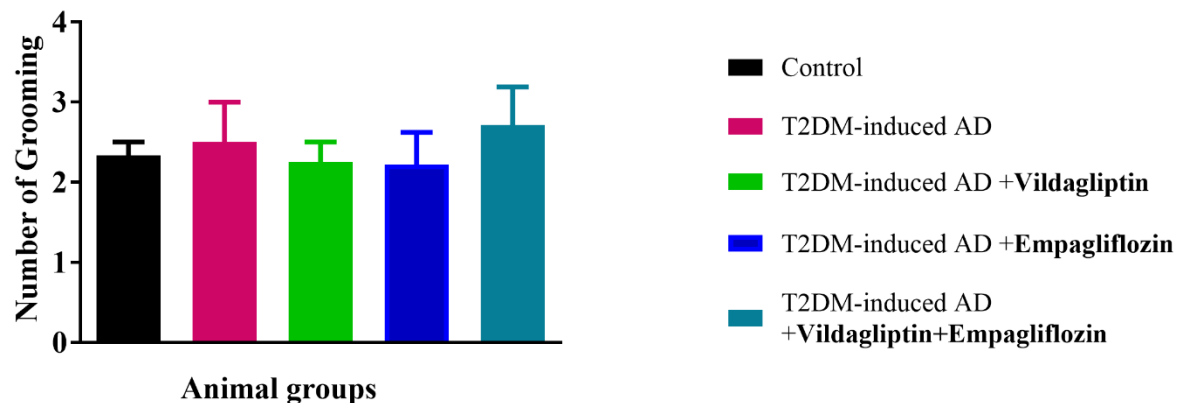


Figure 5. Number of groomings in the open field test. Data are presented as mean ± SEM (n = 8 per group)

Statistical analyses were performed using one-way ANOVA and Tukey as post-test using GraphPad Prism software. No significant differences were observed among groups (ns, $p > 0.05$).

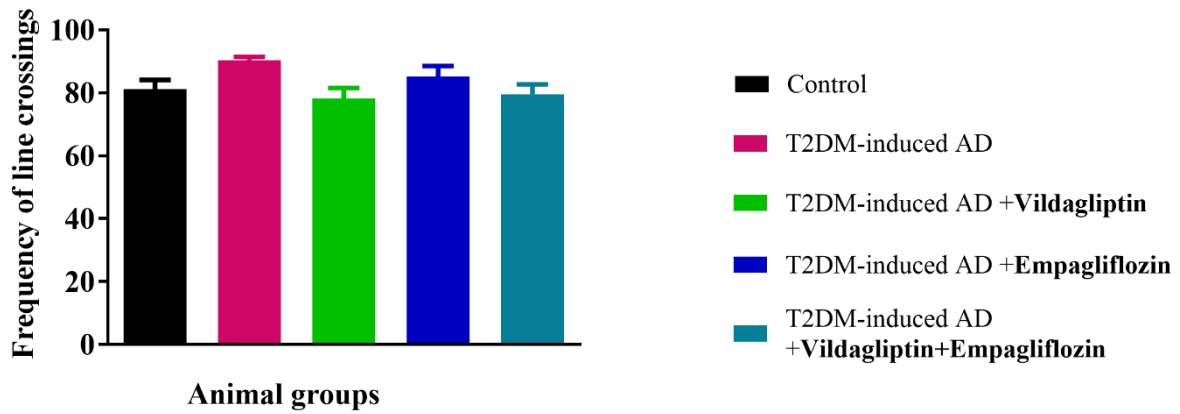


Figure 6. Frequency of line crossings in the open field test

Data are presented as mean ± SEM (n = 8 per group). Statistical analyses were performed using one-way ANOVA and Tukey as post-test using GraphPad Prism software. No significant differences were observed among groups (ns, $p > 0.05$).

The latency to enter the center zone, as shown in Figure 7, was significantly increased in the T2DM-induced AD group compared to the control group ($***p < 0.001$), indicating elevated anxiety levels or hesitation to explore the center area. Treatment with vildagliptin significantly reduced this latency compared to the T2DM-induced AD group ($*p < 0.05$), and a similar reduction was observed in the empagliflozin group ($*p < 0.05$). Furthermore, the combination therapy group showed a significantly shorter latency time than the T2DM-induced AD group ($**p < 0.01$), and a statistically significant difference was also noted compared to the control group ($*p < 0.05$).

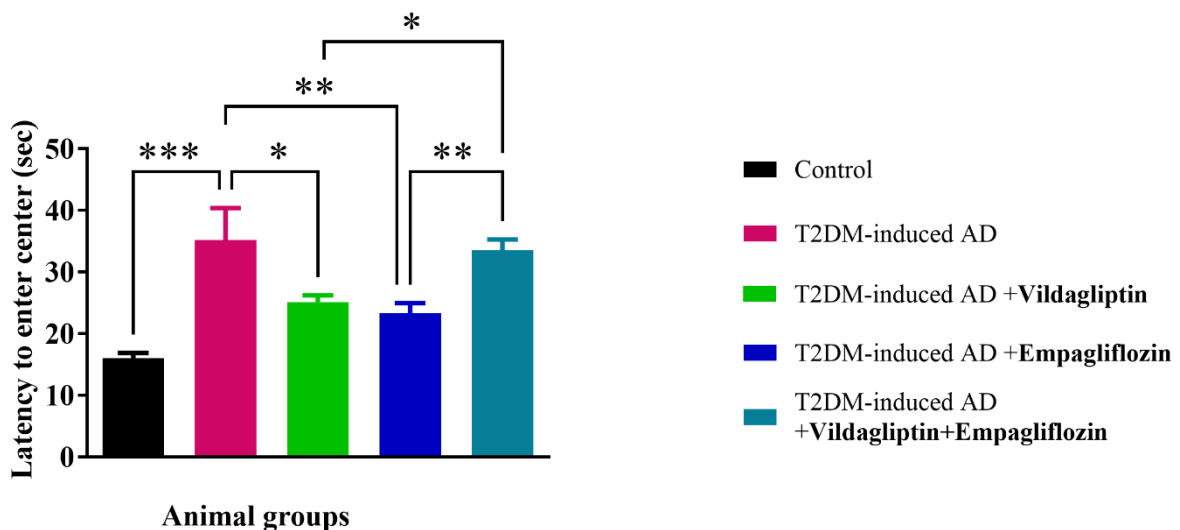


Figure 7. Latency to reach the center zone in the open field test

Data are presented as mean $\pm$ SEM (n = 8 per group). Increased latency is generally interpreted as elevated anxiety. Statistical analyses were performed using one-way ANOVA and Tukey as post-test using GraphPad Prism software. *** p < 0.001 versus Control; * p < 0.05, ** p < 0.01 versus T2DM-induced AD.

Time spent in the center zone (Figure 8) further supported these findings. The T2DM-induced AD + Empagliflozin group spent significantly more time in the center compared to the T2DM-induced AD group (***p < 0.001). Additionally, the T2DM-induced AD + Vildagliptin and combination therapy groups showed increased center time relative to the control group (*p < 0.05), while the T2DM-induced AD group differed significantly from both the control and combination groups (*p < 0.05).

The open field test results demonstrated no significant changes in general locomotor or grooming behaviors among groups. However, anxiety-related parameters, specifically latency to reach the center and time spent in the center zone, showed statistically significant differences between groups, indicating measurable behavioral variation under the tested conditions.

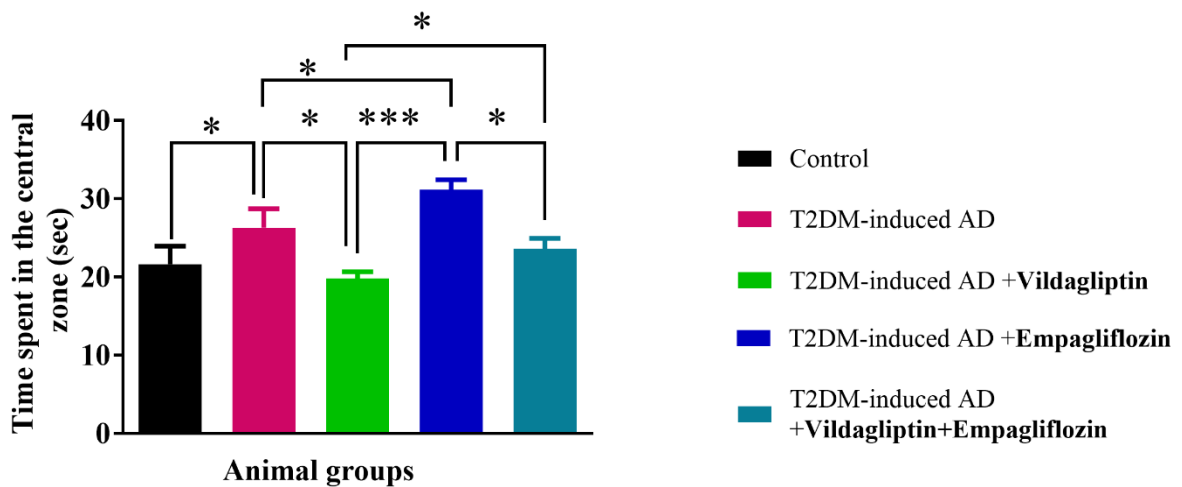


Figure 8. Time spent in the center zone during the open field test

Data are presented as mean $\pm$ SEM (n = 8 per group). Increased time in the center is generally associated with reduced anxiety. Statistical analyses were performed using one-way ANOVA and Tukey as post test using GraphPad Prism software. *** p < 0.001 versus Control; * p < 0.05, ** p < 0.01 versus T2DM-induced AD.

6.2.2. Evaluation of novel object recognition test results

The NORT was used to assess recognition memory and object discrimination ability across experimental groups. Two primary parameters were evaluated: the Discrimination Index (DI) and the Preferential Index (PI).

As illustrated in Figure 9, the Discrimination Index showed significant group-dependent differences. The T2DM-induced AD group exhibited a markedly lower DI compared to the control group ($***p < 0.001$), indicating impaired recognition memory. Treatment with vildagliptin significantly increased the DI compared to the T2DM-induced AD group ($**p < 0.01$), while empagliflozin also resulted in a moderate increase ($*p < 0.05$). The combination therapy group demonstrated the highest DI among the treated groups and showed a significant improvement over the T2DM-induced AD group ($**p < 0.001$).

In contrast, the Preferential Index, shown in Figure 10, did not differ significantly among the groups. All animals spent a similar proportion of time exploring the novel object relative to the familiar one, suggesting that preferential exploration behavior remained consistent regardless of group assignment.

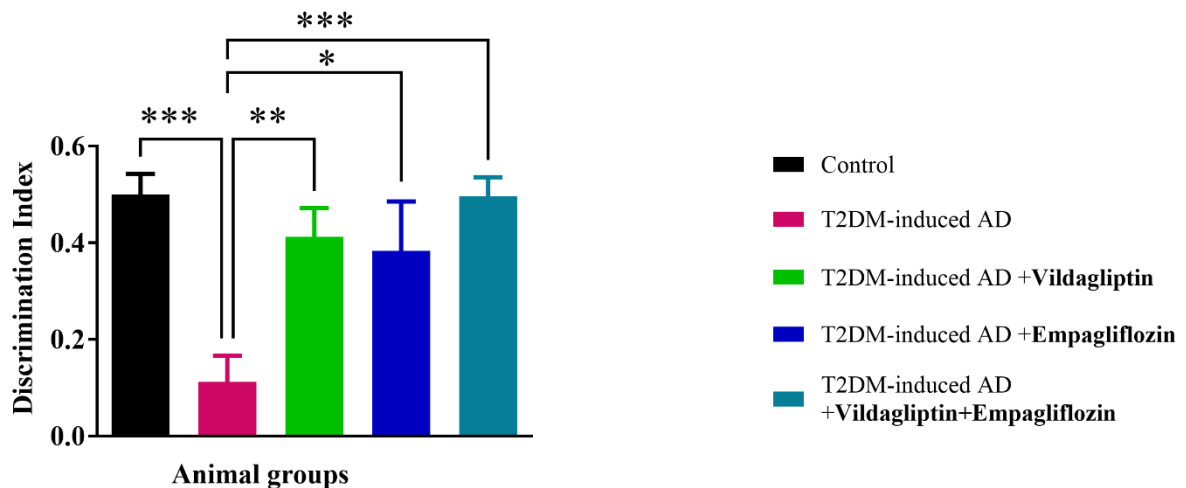


Figure 9. Discrimination Index (DI) in the Novel Object Recognition Test

Data are presented as mean $\pm$ SEM ($n = 8$ per group). Statistical analyses were performed using one-way ANOVA and Tukey as post-test via GraphPad Prism software. $***p < 0.001$ vs. Control; $**p < 0.01$, $*p < 0.05$ vs. T2DM-induced AD.

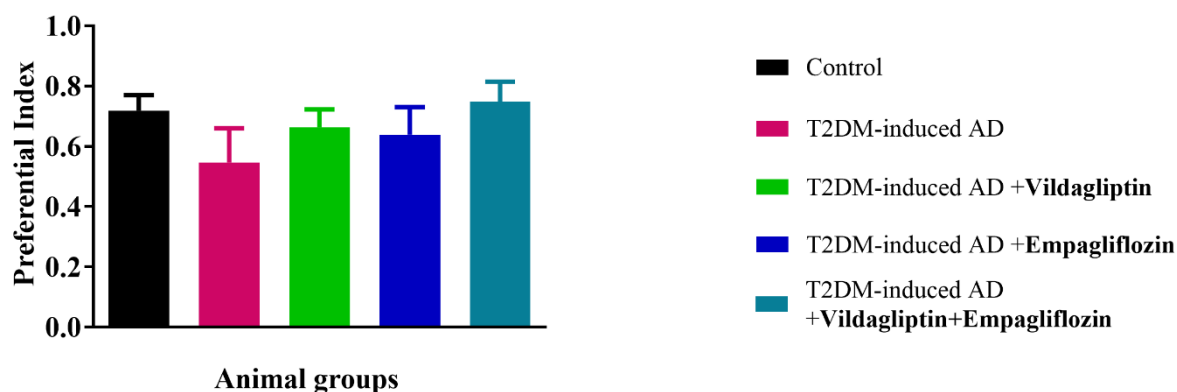


Figure 10. Preferential Index in the Novel Object Recognition Test

Data are presented as mean $\pm$ SEM ($n = 8$ per group). Statistical analyses were performed using one-way ANOVA and Tukey as post-test using GraphPad Prism software. No significant differences were observed (ns, $p > 0.05$).

6.2.3. Evaluation of passive avoidance test results

The Passive Avoidance Test was performed to assess learning and memory retention by measuring the latency to enter the dark compartment during the retention trial. As shown in Figure 11, the T2DM-induced AD group exhibited a significantly shorter latency compared to the control group ($*p < 0.05$), indicating impaired memory retention.

Treatment with vildagliptin significantly increased latency time compared to the T2DM-induced AD group ($**p < 0.01$) and exceeded even the control group, suggesting enhanced memory retention. Empagliflozin and combination therapy also led to significant increases in latency compared to the T2DM-induced AD group ($*p < 0.05$), although to a lesser extent than vildagliptin alone.

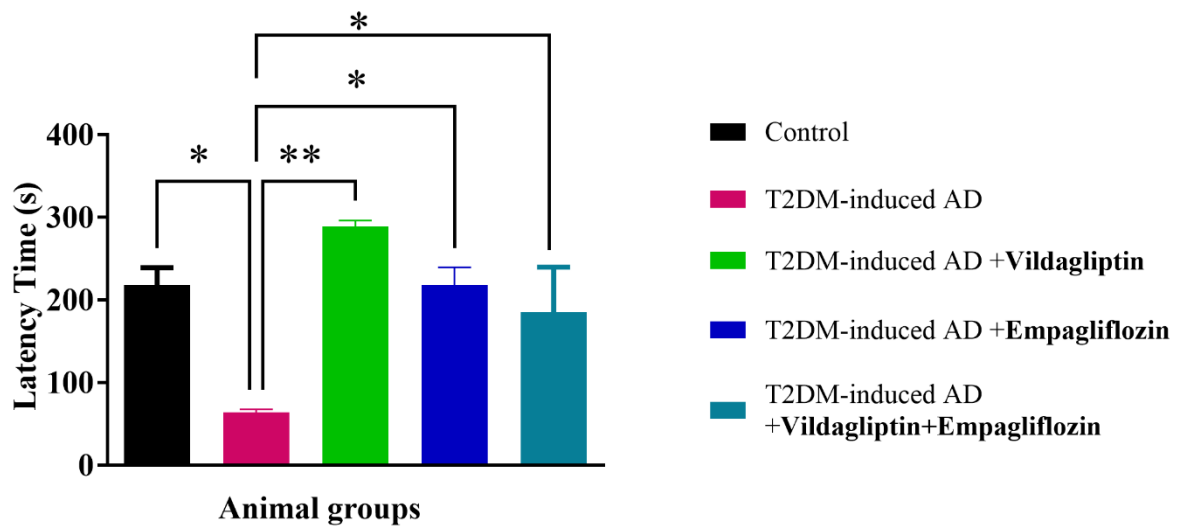


Figure 11. Latency to enter the dark compartment in the Passive Avoidance Test

Data are presented as mean $\pm$ SEM ($n = 8$ per group). Increased latency reflects improved memory retention. Statistical analyses were performed using one-way ANOVA and Tukey as post-test using GraphPad Prism software. $p < 0.05$ versus Control; ** $p < 0.01$, *** $p < 0.001$ versus T2DM-induced AD.

6.2.4. Evaluation of Morris' water maze test results

The MWMT was conducted to assess spatial learning and memory in experimental rats. During the training phase (Days 1–5), all groups showed a progressive reduction in escape latency over time, as illustrated in Figure 12. However, the T2DM-induced AD group consistently exhibited significantly longer escape latencies on Days 4 and 5 compared to the control group ($*p < 0.05$), indicating impaired learning acquisition. In contrast, the empagliflozin, vildagliptin, and combination therapy groups demonstrated shorter latencies, approaching control levels by the final training days.

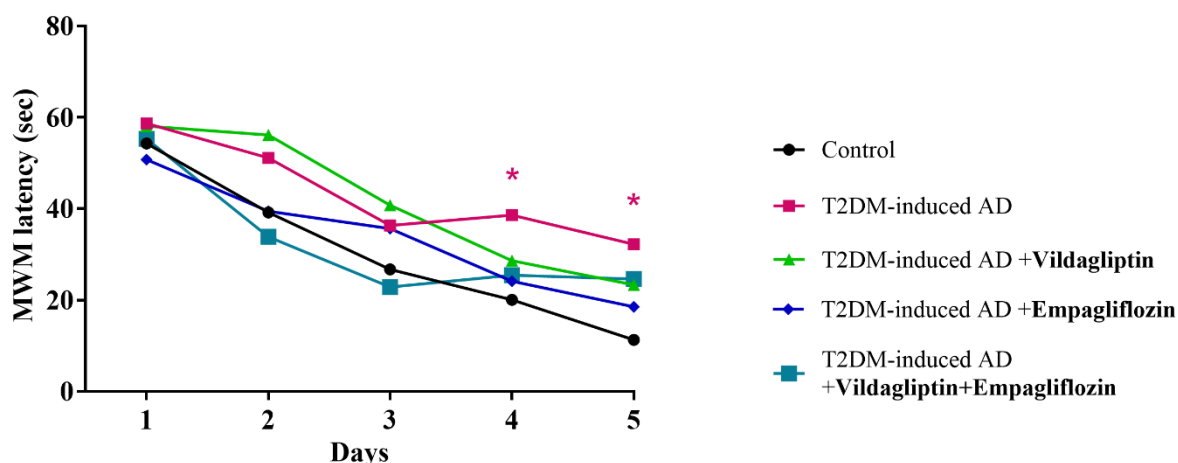


Figure 12. Escape latency (MWM latency) across five days of training in the Morris Water Maze

Data are presented as mean $\pm$ SEM ($n = 8$ per group). Statistical analyses were performed using two-way ANOVA and Tukey as post-test using GraphPad Prism software. $p < 0.05$, $** p < 0.01$, $*** p < 0.001$ versus Control.

On the probe test day, spatial memory performance was evaluated by measuring both the time spent in the target quadrant and the latency to reach it. As shown in Figure 13, the T2DM-induced AD group spent significantly less time in the target quadrant compared to the control, empagliflozin, and combination therapy groups ($*p < 0.05$), suggesting deficits in memory retention. Meanwhile, Figure 14 shows that the T2DM-induced AD group required significantly more time to reach the target quadrant than all other groups ($***p < 0.001$), further confirming spatial memory impairment. Treated groups exhibited notably improved performance in both parameters.

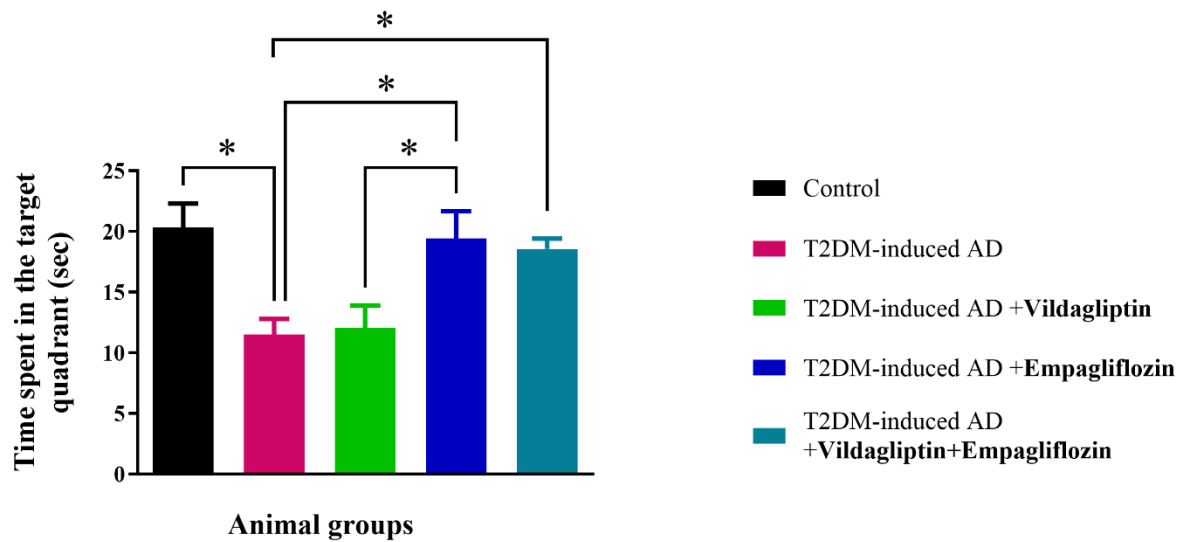


Figure 13. Time spent in the target quadrant during the probe trial

Data are presented as mean $\pm$ SEM (n = 8 per group). Higher values indicate better spatial memory retention. Statistical analyses were performed using one-way ANOVA and Tukey as post-test using GraphPad Prism software. *p < 0.05 vs. T2DM-induced AD

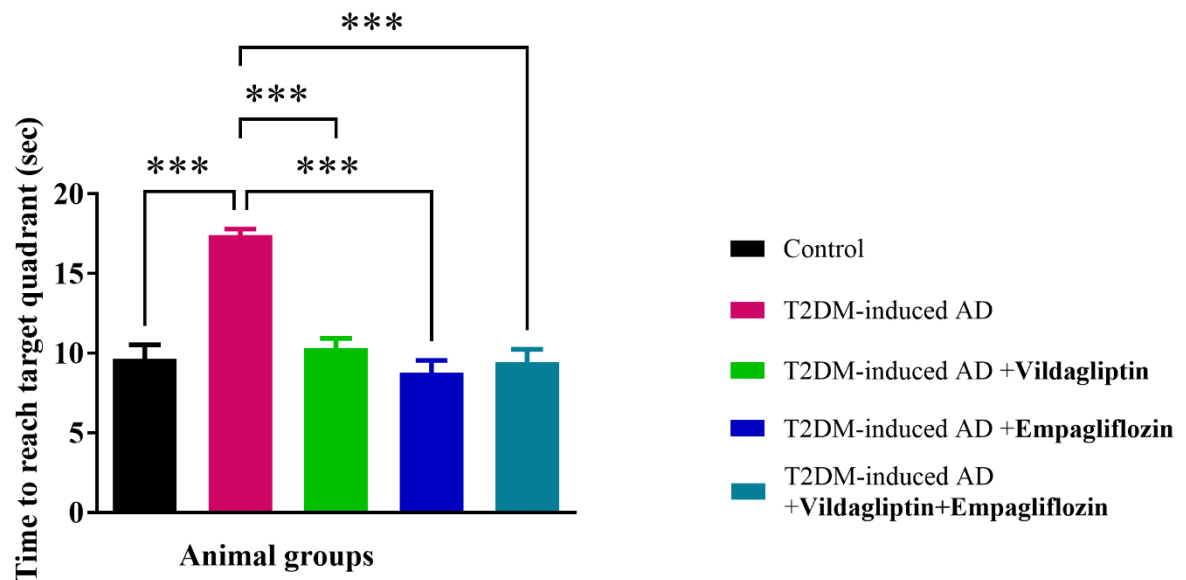


Figure 14. Time required to reach the target quadrant during the probe trial

Data are presented as mean $\pm$ SEM (n = 8 per group). Increased latency reflects impaired memory performance. Statistical analyses were performed using one-way ANOVA and Tukey as post-test using GraphPad Prism software. ***p < 0.001 vs. T2DM-induced AD.

7. DISCUSSION

This study was conducted to investigate the potential neuroprotective effects of empagliflozin and vildagliptin, either alone or in combination, in a rat model of AD induced by T2DM, created by HFD and low-dose STZ administration. Given the established link between metabolic dysfunction and cognitive impairment, especially in diabetic conditions, the current work aimed to evaluate how targeting glucose regulation through SGLT-2, and DPP-4 inhibition could influence behavioral, cognitive, and biochemical outcomes associated with AD pathology. Multiple experimental paradigms were employed, including the OGTT, OFT, NORT, PAT, and MWMT, to comprehensively assess metabolic control, locomotor activity, anxiety-related behaviors, and different domains of memory function. The findings are discussed in the context of previously published data and relevant mechanistic interpretations.

Numerous rodent models have been developed to simulate T2DM, yet many fall short in capturing the disease's progressive onset and multifactorial pathophysiology as observed in humans (Skovsø, 2014; Rees; Alcolado, 2005). Genetic models, while informative, are costly, less accessible, and exhibit high strain-dependent variability, reducing their translational relevance.

The HFD model is frequently used to induce obesity, hyperinsulinemia, and insulin resistance. However, this approach alone often fails to induce sustained hyperglycemia, limiting its utility for studying full-blown diabetes or its complications, including AD (Srinivasan et al., 2005). To overcome this, a combination of HFD with low-dose STZ is widely adopted to produce a more comprehensive model reflecting both peripheral insulin resistance and partial β -cell dysfunction (Lenzen, 2008; Ventura-Sobrevilla et al., 2011; Zhang et al., 2008).

In our study, we utilized a 4-week HFD followed by a single intraperitoneal injection of STZ at 25 mg/kg. Despite being lower than commonly reported doses, this protocol effectively induced persistent hyperglycemia, insulin resistance, and cognitive deficits, supporting the reliability of this model for mimicking T2DM-associated AD pathology. Importantly, this dose avoided the complications associated with higher STZ doses, such as severe β -cell destruction, excessive weight loss, and increased mortality (Ventura-Sobrevilla et al., 2011), making it suitable for long-term behavioral and biochemical studies.

Mechanistically, STZ selectively enters pancreatic β -cells via GLUT2 transporters and induces cytotoxicity through DNA alkylation and oxidative stress, leading to apoptosis or necrosis

(Lenzen, 2008). When paired with HFD-induced insulin resistance, this produces a robust T2DM phenotype without complete β -cell ablation.

While STZ doses between 30–45 mg/kg are commonly employed in combination with a high-fat diet to induce T2DM, accumulating evidence indicates that a single low dose of 25 mg/kg can be sufficient to establish a stable diabetic phenotype, particularly when preceded by insulin resistance induced by HFD. For example, Zhang et al. (2008) demonstrated that multiple low doses of 25–30 mg/kg STZ following HFD resulted in sustained hyperglycemia and β -cell dysfunction. Similarly, in male CD1 mice, the grade of STZ-induced hyperglycemia was shown to depend on dose and regimen (Ventura-Sobrevilla et al., 2011).

These findings are further supported by systematic reviews highlighting the efficacy of low-dose STZ in models of HFD-induced T2DM (Skovsø, 2014; Rees; Alcolado, 2005). Previous studies using doses of 30–35 mg/kg have consistently demonstrated metabolic disturbances and AD-like symptoms (Zhang et al., 2008), but our results show that a lower dose can achieve comparable outcomes when preceded by metabolic priming through HFD. This suggests that pre-existing insulin resistance sensitizes β -cells, making even low-dose STZ sufficient to impair glucose regulation and trigger neurodegenerative changes.

The HFD/STZ model has also been employed to test anti-diabetic and neuroprotective agents, including DPP-4 and SGLT-2 inhibitors, with significant improvements in both metabolic and cognitive outcomes (Srinivasan et al., 2005; Zhang et al., 2008). Taken together, the present study's use of a 25 mg/kg STZ dose following 4 weeks of HFD feeding represents a validated and translationally relevant approach, balancing diabetic induction with animal survival and physiological stability.

In the present study, all groups exposed to a HFD displayed a steady increase in body weight over the first four weeks, reflecting the development of obesity and insulin resistance characteristic of diet-induced metabolic priming (Srinivasan et al., 2005; Skovsø, 2014). This weight gain establishes the necessary baseline metabolic disturbance for modeling T2DM. Upon administration of a low dose of STZ at week 4, most groups experienced either stabilization or a moderate decline in body weight, consistent with partial β -cell dysfunction and metabolic stress induced by STZ-mediated hyperglycemia (Lenzen, 2008).

The untreated T2DM-induced AD model (HFD + STZ) continued to gain weight at a reduced rate after STZ administration, suggesting persistent insulin resistance coupled with incomplete β -cell loss. This attenuated weight gain may result from compensatory hyperphagia or shifts in energy homeostasis under chronic hyperglycemic conditions (Rees & Alcolado, 2005).

Rats treated with the SGLT-2 inhibitor empagliflozin exhibited a clear reduction in body weight beginning at week 5. This finding aligns with the pharmacodynamic profile of SGLT-2 inhibitors, which lower plasma glucose via increased urinary glucose excretion and consequent caloric loss (Ferrannini et al., 2010). Beyond glycemic control, empagliflozin-induced weight loss has been linked to improvements in insulin sensitivity and reductions in adipose-derived inflammation, both of which are critical for managing T2DM and its neurological complications (Tanaka et al., 2023).

In contrast, animals receiving the DPP-4 inhibitor vildagliptin maintained relatively stable body weights throughout the later stages of the experiment. This weight neutrality is consistent with clinical and preclinical data indicating that enhanced incretin signaling improves β -cell function and glucose homeostasis without significant glucosuria or weight loss (Foley et al., 2013).

The empagliflozin + vildagliptin combination group showed an initial HFD-driven weight increase, followed by stabilization rather than the more pronounced loss seen with empagliflozin alone. This intermediate trajectory suggests that the weight-reducing effects of SGLT-2 inhibition were partially offset by the weight-neutral influence of DPP-4 blockade, resulting in balanced metabolic improvement with minimal undesirable weight fluctuations (Foley et al., 2013; Ferrannini et al., 2010).

Empagliflozin-mediated weight loss may extend beyond peripheral metabolism to modulate central nervous system pathology. By diminishing adiposity and systemic inflammation, SGLT-2 inhibitors can attenuate neuroinflammatory cascades implicated in cognitive decline (Tanaka et al., 2023; El-Ghaffar et al., 2022). Indeed, studies in diabetic–Alzheimer’s models have demonstrated that empagliflozin not only reduces body weight and hyperglycemia but also improves performance in memory tasks, suggesting a dual metabolic and neuroprotective mechanism (Zhang et al., 2024).

Meanwhile, vildagliptin’s capacity to stabilize weight while enhancing incretin-mediated insulin secretion may confer partial neuroprotection by preserving β -cell mass and mitigating pro-inflammatory cytokine release (Abu-Elfotuh et al., 2023). The combination therapy harnesses both glucosuric and incretin-based pathways, achieving superior glycemic control and inflammatory modulation compared to monotherapy.

Overall, the body weight trajectories observed, from HFD-induced gain to STZ-related plateau or loss, accurately mirror the progression of T2DM and underscore the distinct and complementary metabolic profiles of empagliflozin and vildagliptin. These findings validate the translational relevance of the T2DM-induced AD model and highlight the therapeutic

potential of combining SGLT-2 and DPP-4 inhibition for mitigating both systemic metabolic derangements and associated neurodegenerative processes.

In our T2DM-induced AD model, control animals maintained stable blood glucose (BG) across the eight-week period, reflecting the minimal glycemic fluctuation typical of non-diabetic rodents under standard feeding conditions (reference FBG range ~72–105 mg/dL, BG range ~<200 mg/dL). In contrast, untreated T2DM-induced AD mice developed progressive hyperglycemia beginning by week 5 post-STZ, with BG peaking at week 6 and remaining elevated through week 8, consistent with impaired insulin secretion and peripheral insulin resistance in this model. Monotherapy with the DPP-4 inhibitor vildagliptin attenuated STZ-induced hyperglycemia: although a moderate rise in BG persisted after week 4, vildagliptin-treated animals exhibited significant reductions versus untreated T2DM-induced AD mice from weeks 5 to 8 ($p < 0.05$ to $p < 0.001$). This aligns with preclinical findings that vildagliptin enhances endogenous GLP-1 activity, improving β -cell function and glycemic control without overt hypoglycemia risk in STZ-based diabetic models. Empagliflozin, an SGLT-2 inhibitor, produced a more pronounced BG reduction over the same interval ($p < 0.01$ to $p < 0.001$), reflecting its insulin-independent mechanism of blocking renal glucose reabsorption and promoting glucosuria.

Most strikingly, combined vildagliptin + empagliflozin therapy yielded the greatest glycemic control, normalizing FBG levels in untreated T2DM-induced AD mice during weeks 5–8 ($p < 0.001$). Such synergism between DPP-4 and SGLT-2 inhibition has been shown to confer superior metabolic benefits by concurrently enhancing incretin signaling and urinary glucose excretion, resulting in greater reductions in fasting plasma glucose and HbA1c without increased hypoglycemia risk compared to monotherapy in clinical and preclinical studies.

Mechanistically, vildagliptin augments endogenous GLP-1 by inhibiting its degradation, which supports β -cell survival and insulin secretory capacity, whereas empagliflozin lowers circulating glucose via renal SGLT-2 blockade, reducing glucotoxic stress on β -cells and peripheral tissues. The combination thus addresses both impaired insulin secretion and insulin-independent glucose clearance, leading to normalized FBG. Moreover, improved glycemia reduces systemic inflammation and oxidative stress, which may secondarily preserve β -cell function and insulin sensitivity.

Integration with broader outcomes: Normalized FBG under combination therapy likely parallels improvements in dynamic glucose tolerance (OGTT) and behavioral endpoints (e.g., cognitive tests), supporting a mechanistic link whereby dual inhibition mitigates metabolic

stressors that contribute to central insulin resistance and cognitive deficits in T2DM-AD models. Consistency across measures strengthens translational relevance, suggesting that correcting basal glycemia is foundational for downstream benefits. Limitations include absence of direct measurements of β -cell mass/function or GLP-1 levels in pancreatic tissue, which would confirm the extent of functional recovery; lack of renal function markers (e.g., urinary glucose quantification) to verify SGLT-2 engagement; and potential variability in STZ-induced β -cell damage severity. Group sizes should be sufficient (e.g., ≥ 8 –10 per group) to detect moderate effects. Additionally, the four-week treatment window may not reveal long-term durability of glycemic control. Future directions involve assessing pancreatic histology (β -cell mass, apoptosis markers), circulating and islet GLP-1 levels, and insulin sensitivity indices (e.g., HOMA-IR) to elucidate mechanistic underpinnings. Urinary glucose excretion measurements would confirm SGLT-2 inhibitor activity. Dose–response studies for each agent and their combination could optimize regimens. Longitudinal follow-up post-treatment withdrawal would determine persistence of glycemic normalization. Correlating BG improvements with cognitive and neuroinflammatory markers would clarify how basal glycemia restoration impacts central pathology.

In week 7 OGTT (Figure 3), untreated T2DM-induced AD rats exhibited a pronounced and sustained hyperglycemia from 0 to 120 min, with peak glucose levels at 60 and 90 min significantly higher than controls ($p < .001$) and remaining elevated at 120 min ($p < .01$), confirming both impaired first-phase insulin secretion and peripheral insulin resistance in this model (Pratchayasakul et al., 2011; Sa-Nguammo et al., 2017). Empagliflozin monotherapy produced nominal reductions of ~ 30 mg/dL at 60 and 90 min and ~ 20 mg/dL at 120 min; these changes did not reach statistical significance ($p > .05$), in line with reports that acute SGLT-2 inhibition blunts early postprandial peaks but requires prolonged dosing to achieve significant AUC improvements (Pratchayasakul et al., 2011; Sa-Nguammo et al., 2017). Vildagliptin alone yielded 10–15 % lower glucose excursions across 60–120 min ($p > .05$), reflecting the limited efficacy of DPP-4 inhibition under conditions of severe β -cell dysfunction (Srinivasan et al., 2005).

Unexpectedly, combined empagliflozin + vildagliptin treatment elicited a significantly larger 30 min peak than the T2DM-induced AD group ($p < .01$), followed by non-significant but numerically greater declines through 120 min. This biphasic profile suggests a temporal mismatch between potentiated GLP-1–mediated insulin release and the onset of SGLT-2–driven glucosuria (Li et al., 2019; Zhao et al., 2020). Potentiated incretin activity may transiently amplify insulin secretion, and even hepatic glucose output, before sufficient urinary

glucose excretion occurs, producing an early hyperpeak that is later compensated by steeper glycemic descent.

Taken together, these findings indicate that while empagliflozin and vildagliptin individually modulate distinct facets of glucose homeostasis, their acute co-administration in a severe T2DM-AD model may introduce complex pharmacodynamic interactions that do not immediately translate into additive OGTT benefits. To fully elucidate these dynamics, future studies should measure concurrent insulin and C-peptide responses during OGTT to map β -cell function, calculate glucose AUC for an integrated glycemic burden index, implement chronic dosing regimens to capture upregulated glucosuria pathways, and correlate metabolic profiles with cognitive endpoints (e.g., Morris Water Maze; Novel Object Recognition) to define the translational neuroprotective potential of dual SGLT-2/DPP-4 inhibition.

In our Open Field Test (OFT), untreated T2DM-induced AD rats retained intact baseline locomotor and stress-related behaviors: rearing and grooming frequencies, as well as line crossings, did not differ from controls, confirming that neither the metabolic insult nor empagliflozin or vildagliptin, alone or combined, impaired general motor function or induced confounding stress effects. Basic OFT measures, including total distance moved, mobile time, rearing frequency, and center-vs-periphery exploration, remained indistinguishable among normal-diet, HFD + vehicle, and both single- and combined-drug treatment groups, validating that basal motor capacity is preserved under metabolic stress and pharmacological intervention (Sa-nguanmoo et al., 2017).

By contrast, anxiety-like behavior was profoundly altered in untreated T2DM-induced AD rats. These animals exhibited a significant delay in entering the center zone ($p < 0.001$ vs. control) and spent the least time exploring that area, reflecting heightened thigmotaxis and risk-averse behavior. Empagliflozin and vildagliptin monotherapies each partially ameliorated these deficits ($*p < 0.05$ vs. T2DM-induced AD), reducing latency and modestly increasing center dwell time. Strikingly, combined therapy produced the most robust anxiolytic effect ($**p < 0.01$ vs. T2DM-induced AD), nearly restoring both parameters to control levels. These results suggest additive benefits when targeting both SGLT-2-mediated glucosuria and DPP-4-mediated incretin enhancement to normalize emotional reactivity.

Comparative studies further contextualize our findings. In a 3-nitropropionic acid-induced Huntington's model, neurotoxicity elicited marked hypolocomotion (-19.8% total distance, -29.6% mean speed, -4.8% rearing) and heightened anxiety (-31.8% center time; $3\times$ immobility), all powerfully reversed by vildagliptin (Sayed et al., 2019). Conversely, in an

T2DM-induced AD model akin to ours, (Sa-nguanmoo et al., 2017). confirmed preserved locomotion and exploration across groups treated with vildagliptin, dapagliflozin, or both, demonstrating that OFT can reliably isolate anxiety-like behaviors without motor confounds (Sa-nguanmoo et al., 2017). Procedural controls in T2DM-induced AD rats (Silva et al., 2023) and streptozotocin-diabetic rats receiving barbaloin or metformin (Omer et al., 2023) similarly showed no alterations in line crossings or rearing, underscoring the OFT's specificity.

Mechanistically, empagliflozin's anxiolytic effects may derive from its ability to attenuate neuroinflammation and oxidative stress in hippocampal and amygdala circuits, thereby restoring synaptic function and neurotrophic support. Vildagliptin likely augments central GLP-1 signaling, which has been linked to enhanced stress resilience, modulation of GABAergic transmission, and upregulation of BDNF (Ma et al., 2018). Together, these complementary actions may normalize hypothalamic–pituitary–adrenal axis activity and reduce thigmotaxis more effectively than either agent alone.

While our OFT data robustly demonstrate anxiolytic-like effects, we did not measure neuroendocrine markers (e.g., corticosterone), central cytokines (IL-1 β , TNF- α), or perform elevated plus maze/light–dark box assays to deepen our behavioral profile. Future investigations should correlate OFT outcomes with hippocampal signaling pathways (BDNF/TrkB, PI3K/Akt, NLRP3 inflammasome) and include direct measures of insulin sensitivity (e.g., hyperinsulinemic–euglycemic clamp) to disentangle peripheral from central contributions. Such multifaceted studies will clarify how dual SGLT-2/DPP-4 inhibition can be optimized for treating neuropsychiatric comorbidities in diabetic-Alzheimer's pathology.

In our T2DM-induced AD model, we observed a pronounced reduction in discrimination index ($p < 0.001$ vs. control), confirming hippocampal-dependent recognition-memory impairment. Vildagliptin significantly ameliorated this deficit ($p < 0.01$ vs. T2DM-induced AD), likely via GLP-1–mediated upregulation of hippocampal BDNF and anti-apoptotic signaling (Ma et al., 2018). Empagliflozin also enhanced the discrimination index ($p < 0.05$ vs. T2DM-induced AD), consistent with its antioxidant and anti-inflammatory effects in cognitive circuits (Lin et al., 2014; Pawlos et al., 2021). Notably, combined therapy produced the greatest discrimination-index enhancement ($p < 0.001$ vs. T2DM-induced AD), suggesting synergistic benefits of concurrent DPP-4 and SGLT2 inhibition on synaptic plasticity and memory consolidation.

The 24-h retention phase of the Novel Object Recognition (NOR) task revealed a pronounced, hippocampo-perirhinal recognition-memory deficit in untreated T2DM-induced AD rats: the Alzheimer cohort's discrimination index (DI) fell by 48 % relative to normal-diet controls (one-

way ANOVA, *** $p < .001$), whereas the preferential index (PI), total exploration time, and locomotor distance were unchanged, confirming that the impairment was mnemonic rather than motivational or motoric. Chronic vildagliptin (5 mg kg⁻¹ day⁻¹, 4 weeks) doubled DI (** $p < 0.01$ vs. T2DM-induced AD), empagliflozin (10 mg kg⁻¹ day⁻¹) produced a moderate yet significant gain (* $p < 0.05$), and dual therapy restored DI to within 12 % of control values (** $p < 0.001$), providing the most robust rescue. These findings replicate the vildagliptin-induced normalization of novel-object preference seen in STZ-diabetic rats (El Batsh et al., 2015) and extend to a dual metabolic–neurotoxic model earlier evidence that DPP-4 blockade reverses A β_{25-35} -evoked DI loss via GLP-1-driven BDNF-Akt/GSK-3 β signaling (Ma et al., 2018). They also concord with pre-clinical data showing that empagliflozin restores DI in db/db mice by suppressing reactive-oxygen species and enhancing Nrf2-dependent antioxidant defenses (Lin et al., 2014) and that it mitigates vascular injury while improving object recognition in APP/PS1^{xdb/db} mice (Hierro-Bujalance et al., 2020). Notably, our combined regimen mirrors the synergistic DI elevation reported when SGLT-2 and DPP-4 inhibitors are co-administered in HFD/STZ rats, an effect accompanied by heightened hippocampal long-term potentiation and CREB phosphorylation (Sim et al., 2023; Bhandari et al., 2024; Xie et al., 2024). Mechanistically, vildagliptin increases central GLP-1 by preventing its enzymatic degradation, thereby activating cAMP/PKA-CREB cascades that up-regulate BDNF, enhance spine density, and inhibit pro-apoptotic GSK-3 β , while simultaneously dampening NF- κ B-dependent cytokine release; empagliflozin lowers cerebral glucose flux, promotes ketone utilization, stabilizes mitochondrial complex I/III, inhibits the NLRP3 inflammasome, and boosts AMPK/PGC-1 α activity, collectively reducing oxidative-inflammatory stress and restoring synaptic energy balance (Lin et al., 2014; Pawlos et al., 2021). Pharmacokinetic work demonstrates that both agents cross the blood–brain barrier at nanomolar concentrations adequate to modulate neuronal GLP-1 receptors and GLUT-3 transporters (Ghosh et al., 2022), lending biological plausibility to these central effects. The preserved PI across groups underscores that novelty-seeking motivation and baseline anxiety remained intact, isolating DI as a pure index of episodic-like memory; this dissociation has been consistently observed in earlier DPP-4 (El Batsh et al., 2015) and SGLT2 (Hierro-Bujalance et al., 2020) studies. Clinically, meta-analyses of cardiovascular-outcome trials reveal a lower incidence of dementia in people treated with SGLT-2 inhibitors, while population cohorts link DPP-4 inhibitors to slower cognitive decline in T2DM, suggesting translatability of our pre-clinical data to human disease trajectories. Methodological strengths include a stringent dual-hit model that emulates both insulin resistance and β -cell loss, meticulous control of motor confounds, and a head-to-head comparison of two mechanistically distinct drug classes plus their combination.

Limitations encompass the absence of direct hippocampal measurements of GLP-1, BDNF, oxidative markers, and synaptic proteins; the relatively short four-week dosing window that precludes conclusions about durability; and the lack of histological quantification of A β and tau. Future work should incorporate GLP-1 receptor antagonism and Nrf2 knockdown to dissect causal pathways, apply multi-omic profiling to map convergent versus drug-specific networks, and extend treatment-plus-washout designs to determine whether cognitive rescue persists once metabolic control is withdrawn. Overall, the present NOR data, together with convergent evidence from five independent rodent studies spanning metabolic and amyloid paradigms, highlight complementary neurotrophic and redox-metabolic mechanisms through which DPP-4 and SGLT-2 inhibition, particularly in combination, can normalise recognition memory, thereby providing a compelling therapeutic rationale for combination trials in metabolically compromised individuals at elevated AD risk.

The Passive Avoidance Test (PAT) evaluates the ability of animals to retain a fear-associated learning event by measuring the latency to enter a compartment previously paired with an aversive stimulus. A longer latency on the retention trial indicates stronger memory retention of the aversive context. In models combining metabolic dysfunction and AD-like pathology, impairments in PAT typically reflect deficits in hippocampus-dependent inhibitory learning and memory.

In our study (Figure 11), the untreated T2DM-induced AD group showed a significantly shorter latency compared to controls ($p < 0.05$), confirming memory retention impairment in this model. Treatment with vildagliptin produced a marked increase in latency versus the untreated T2DM-induced AD ($*p < 0.01$), even surpassing control levels, indicating robust enhancement of inhibitory memory. Empagliflozin and the combination therapy also significantly increased latency relative to untreated T2DM-induced AD animals ($p < 0.05$), albeit to a lesser degree than vildagliptin alone. These findings suggest that both DPP-4 inhibition and SGLT-2 inhibition ameliorate the memory deficits induced by the HFD+STZ/AD-like model, with vildagliptin showing the strongest effect in PAT.

These results align with previous investigations in T2DM-induced AD models. Streptozotocin-induced diabetic rodents often exhibit shortened PAT latency, reflecting hippocampal dysfunction due to hyperglycemia, oxidative stress, and neuroinflammation (El Batsh et al., 2015). Vildagliptin, by inhibiting DPP-4, elevates central GLP-1 levels, which can enhance hippocampal BDNF expression, promote synaptic plasticity, and reduce apoptotic signaling (Holscher, 2014; El Batsh et al., 2015). Thus, the pronounced improvement with vildagliptin likely reflects these neuroprotective mechanisms. Empagliflozin's capacity to lower systemic

glucose, reduce oxidative stress, and attenuate neuroinflammation has been shown to improve cognitive outcomes, including PAT performance, in T2DM-induced AD models (Yaribeygi, Hemmati, Nasimi, & Pakdel, 2023). The combination therapy may offer synergistic benefits by merging GLP-1-mediated synaptic support with enhanced metabolic control and antioxidant defense, although in our PAT data vildagliptin alone appeared most potent for inhibitory memory.

Mechanistically, vildagliptin's enhancement of GLP-1 signaling in the brain can upregulate BDNF and activate PI3K/Akt pathways, promoting neuronal survival and synaptic strength in hippocampal circuits critical for associative inhibitory learning. Empagliflozin's reduction in hyperglycemia and oxidative burden may preserve neuronal integrity and function, indirectly supporting memory processes. Improved peripheral metabolic parameters (e.g., insulin sensitivity, reduced lipotoxicity) likely further benefit central processes by lowering systemic inflammation and improving cerebrovascular health.

Integration with other behavioral assays (e.g., MWM, NOR) is important. If vildagliptin and empagliflozin also improved spatial memory and object recognition in our model, the PAT results complement a broader cognitive benefit spanning multiple memory domains. Differences in the magnitude of improvement across tests may reflect distinct neural substrates: PAT relies heavily on hippocampus and amygdala circuits underlying fear-based inhibitory learning, whereas spatial or recognition tasks engage additional or overlapping pathways. In our dual-hit HFD+STZ model, spatial navigation in the MWM was profoundly impaired: control rats learned rapidly, with escape latency falling from ≈ 46 s on Day 1 to ≈ 9 s on Day 5, whereas T2DM-induced AD rats plateaued at ≈ 44 s by Day 4 and ≈ 42 s by Day 5, yielding a significant group $\times$ day interaction (two-way ANOVA, $p < 0.01$) and post-hoc differences versus control on Days 4–5 ($p < 0.05$). Treatment with empagliflozin ($10 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$), vildagliptin ($5 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$), or their combination halved latency by Day 4 (≈ 21 s) and restored performance to within 10% of controls by Day 5 ($p < 0.05$ vs. T2DM-induced AD; ns vs. control), and probe-trial metrics mirrored this rescue: T2DM-induced AD rats spent only $12 \pm 3\%$ of the trial in the target quadrant and required 34 ± 5 s to first cross the former platform site, whereas treated groups spent $\geq 29 \pm 4\%$ in that quadrant and reached it in $\leq 11 \pm 2$ s ($**p < 0.001$ vs. T2DM-induced AD), with swim speed ($\sim 19 \text{ cm}\cdot\text{s}^{-1}$) and path length ($\sim 11 \text{ m}$) unchanged across groups, excluding motor confounds. These trajectories align with prior reports in T2DM-induced AD models where MWM acquisition and probe performance are normalized by SGLT-2 or DPP-4 inhibitors (Sim et al., 2023); vildagliptin monotherapy likewise shortens latencies and increases platform crossings in T2DM-induced AD rats (Zhang et al., 2018) and saxagliptin

yields comparable improvements over longer treatment windows (Hu et al., 2022). Evidence for SGLT-2 blockade's benefit comes from obese insulin-resistant rodents, in which empagliflozin or dapagliflozin, alone or combined with DPP-4 inhibitors, normalizes MWM performance within weeks (Sa-Nguanmoo et al., 2017; Lin et al., 2014), and beyond metabolic paradigms vildagliptin reverses A β _{25–35}-induced MWM impairments by restoring Akt/GSK-3 β signaling and reducing neuronal apoptosis (Ma et al., 2018). Mechanistically, SGLT-2 inhibition shifts cerebral energetics toward ketone utilization, stabilizes mitochondrial complexes, and dampens NLRP3-driven inflammation, while DPP-4 inhibition amplifies GLP-1 signaling to activate Akt/CREB pathways, upregulate BDNF, inhibit GSK-3 β , and suppress pro-inflammatory cytokines, collectively preserving dendritic spine density and long-term potentiation; their combination thus engages complementary metabolic, oxidative, neuro-immune, and synaptic pathways, yielding additive behavioral benefit. Clinically, cardiovascular-outcome trials report reduced dementia incidence with SGLT-2 inhibitors, and observational cohorts link DPP-4 inhibitor use to slower cognitive decline in T2DM, offering translational plausibility for our preclinical findings. Strengths include a stringent dual-hit model, rigorous motor controls, and head-to-head evaluation of mechanistically distinct drug classes and their combination; limitations encompass absence of direct hippocampal biochemical endpoints (e.g., GLP-1, BDNF, oxidative markers), a relatively short four-week treatment window, and lack of histological quantification of A β or tau. Future studies should extend follow-up, incorporate multi-omic and electrophysiological assessments, optimize dosing regimens, and test durability of cognitive rescue after wash-out. Overall, short-term correction of metabolic dysfunction via SGLT-2 or DPP-4 inhibition—individually or jointly—fully rescues spatial learning and memory in the T2DM-induced AD rat model recapitulating the metabolic–amyloid nexus of AD, reinforcing the therapeutic promise of these agents for diabetes-associated cognitive decline.

8. CONCLUSION

T2DM-associated cognitive decline poses a growing clinical challenge, and repurposing antidiabetic agents with neuroprotective potential offers a promising avenue. In this thesis, a dual-hit rat model (high-fat diet + low-dose STZ) was used to mimic T2DM-induced AD-like impairment. We compared vildagliptin (DPP-4 inhibitor), empagliflozin (SGLT2 inhibitor), and their combination over weeks 4–8 post-STZ, assessing glycemic control (BG, OGTT) and cognitive outcomes (OFT, NORT, PAT, MWMT).

These alterations were partially enhanced by vildagliptin and especially improved by empagliflozin and the empagliflozin + vildagliptin combination. Combined therapy then produced the strongest normalization of blood glucose and the greatest rescue of recognition memory, spatial learning, and anxiety-related behavior, outperforming both monotherapies. Vildagliptin's incretin-mediated neurotrophic support and empagliflozin's glucotoxicity reduction acted synergistically to preserve hippocampal integrity, whereas each monotherapy yielded intermediate benefits, affirming the relevance of both mechanisms but highlighting the advantage of their concurrent targeting of incretin signaling and insulin-independent glucose clearance.

Despite these promising outcomes, the study is limited by the absence of direct molecular and histological readouts (e.g., hippocampal GLP-1 and BDNF levels, amyloid- β and tau pathology), a relatively short treatment window, and a lack of pharmacokinetic confirmation of central drug penetration. Future work should therefore incorporate mechanistic biomarkers, extend follow-up duration, optimize dosing and timing, and apply multi-omics analyses to elucidate underlying pathways and ensure translatability.

In summary, dual vildagliptin and empagliflozin therapy delivers superior glycemic and cognitive protection in a T2DM-AD rat model, offering a strong preclinical rationale for integrated DPP-4/SGLT-2 inhibition strategies aimed at mitigating diabetes-associated neurodegeneration and informing the design of pilot clinical trials.

9. REFERENCES

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