MASTER OF SCIENCE (BIOTECHNOLOGY) [SHANU BHARDWAJ] 2023

A DRUG REPURPOSING APPROACH THROUGH PHARMACOPHORE MODELING AND MOLECULAR DOCKING TO MANAGE ALZHEIMER'S DISEASE VIA GSK-3β MODULATION

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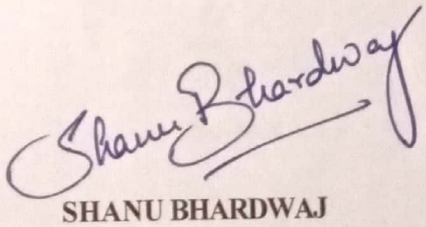
CANDIDATE'S DECLARATION

I, **Shanu Bhardwaj,** (Roll No.: 2K21/MSCBIO/45) of **M.Sc. Biotechnology,** declare that this work which is presented in this Major Project titled "**A drug repurposing approach through pharmacophore modelling and molecular docking to manage Alzheimer's Disease via GSK 3β modulation**" submitted to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirements for the award of the degree of Master of Science, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any degree.



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Shanu Bhardwaj

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CERTIFICATE

To the best of my knowledge, the work titled "**A drug repurposing approach through pharmacophore modelling and molecular docking to manage Alzheimer's Disease via GSK 3β modulation"** has not been submitted anywhere else either in part or in full for any Degree or Diploma at this University or elsewhere. I further certify that the publication and indexing information given by the student is correct.

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ABSTRACT

Alzheimer's Disease is progressive disorder whose pathophysiology and therapeutic status still stands unclear. As of now, all the therapies are confined to symptomatic relief, disease modifying therapies are therefore the need of the hour. Modulation in Wnt cascade has already been linked to varied disorders which include AD and type 2 diabetes mellitus too. The interlink between insulin signaling pathway and Wnt cascade has been well acknowledged through a number of preclinical and clinical studies. This *in silico*-based study is focused upon investigating the link between curative effects of FDA approved anti- diabetic drug and Wnt signaling cascade. We prepared a library consisting of 143 FDA approved antidiabetic medicines with an aim of repurposing them as GSK 3 beta inhibitor, which is present in increased amounts in AD brain. Pharmacophore modelling was performed for these drugs and the lead hits were then subjected to ligand- protein based molecular docking followed by molecular dynamics simulation. ZINC04803471 emerged as a clear winner that can inhibit GSK 3 beta, which leads to beta catenin degeneration and thereby downregulates the canonical Wnt signaling cascade in the AD brain. Although this is just a tip of an iceberg, further in vitro investigation is necessary to validate the effectiveness of the compound.

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LIST OF ABBREVIATIONS

Abbreviation	Full Form
NFTs	Neurofibrillary Tangles
NMDA	N-methyl D-aspartate
NMDAR	N-methyl D-aspartate receptor
ACh	Acetylcholine
AChEIs	Acetylcholinesterase inhibitors
CSF	Cerebrospinal fluid
LTD	Long term depression
LTP	Long Term Potentiation
ChAT	Choline acetyltransferase
Aβ	Amyloid beta
VAChT	Vesicular acetylcholine transporter
EAA	Excitatory amino acid
ROS	Reactive oxygen species
T2D	Type 2 Diabetes
SNPs	Single Nucleotide Polymorphism
LRP-5	Low density lipoprotein related protein 5
AD	Alzheimer's disease
FDA	Food and Drug Administration
GSK-3β	Glycogen synthase kinase 3 beta
TCF/LEF	T-cell factor/lymphoid enhancer factor
LRP6	Low-density lipoprotein receptor-related protein 6
RMSF	Root-mean-square fluctuation
RMSD	Root-mean-square deviation
AD	Alzheimer's Disease
NBM	nucleus basalis of Meynert

CHAPTER-1

INTRODUCTION

1.1 BACKGROUND

Alois Alzheimer, a German psychiatric for the first time reported about AD which is distinguished by neuritic plaques and NFTs as a consequence of Aβ aggregation [1]. When Alois Alzheimer examined his very first patient, who experienced loss of memory and an alteration in behavior before passing away, he identified an excessive amount of amyloid beta plaques and an enormous degeneration of neurons. He further advocated the illness as an awful condition of the cerebral cortex. Emil Kraepelin primarily in his psychiatry handbook's (8^{th} edition) mentioned this severity as AD [2][3]. The financial burden of AD affects families, people, and the economies, with the expected world-wide expenditure of US$1 trillion per annum. At the moment, no proper treatment for AD is available, however there are therapies that merely provide symptomatic relief. Currently, the estimated number of AD suffering peoples are 50 million and by 2050, the anticipated number of people would be 152 million worldwide.[4]

1.2 Problem Statement

Approximately 24 million individuals are said to be afflicted with Alzheimer's disease throughout the world as of the present time, and by 2050, professionals anticipate that this figure would be quadrupled. However, AD is affecting a large chunk of population, there are currently just two types of drugs that can be used to treat AD.: NMDA antagonists, and cholinesterase inhibitors. The decimation of Ach secreting neurons by a several biochemical processes in AD decreases the mediation of cholinergic signals in the brain. Levels of ACh in the synaptic cleft are elevated as a result of cholinesterase

enzymes being impeded from degrading ACh by AChEIs. [5][6]. While, surplus functioning of NMDAR elevates the influx of Ca^{2+}, which stimulate synaptic damage and apoptosis. The NMDAR inhibitor reinstate the NMDAR glutamate receptor's normal functioning by restraining overactivation, that further suppresses Ca^{2+} influx. Despite the fact that these two classes possess curative properties, they merely serve to treat the signs of AD, and are unable to avert the ailment [7]. However, only a handful of clinical investigations on AD were conducted in the past ten years, and the results were extremely unsuccessful. To modify the etiology of AD and provide efficient therapies, a number of processes have been hypothesized, that involve β-amyloid, aberrant tau protein, neuroinflammation, cholinergic pathway. Therefore, it becomes need of the hour to investigate new therapeutic avenue to mitigate pathogenesis of AD [8].

1.3 Objective of Study

- To curate pharmacophore models of FDA approved anti-diabetic medicines.
- To perform molecular docking of the lead hits obtained from pharmacophore modelling.
- To perform MD simulation analysis of the best docked complex to investigate its therapeutic potential to inhibit GSK 3 beta.

CHAPTER-2

REVIEW OF LITERATURE

2.1Pathobiology of AD

2.1.1 Overview of Amyloid Cascade Hypothesis

In 1892, for the first time Paul Blocq and George Mannesco found Abeta plaques. However, beta amyloid isolated from AD patients after a century was reported by Glenner [9]. In 1991, the amyloid hypothesis/theory reported by John Hardy and David Allsop came into the scene [10]. It is reported that hydrolysis of APP causes Aβ production. Several reports also advocated that due to the hydrolytic action of various secretases (α-, β-, and γ-) by different routes, produces C-terminal fragments from APP [11]. The products which are produced by first non-amyloidogenic pathogenic pathway are neuroprotective and neurotrophic for neurons and these are sAPPα and CTF-α. Similarly other smaller fragments are also produced due to the engagement of secretases such as (α- and γ) under normal circumstances. In case of amyloidogenic pathway β-secretase cleaves APP into CTF-β and then γ-secretase assisted production of varied lengths of Aβ such as Aβ42. Aβ42 is highly prone to plaque formation compared to Aβ40 that bears stronger neurotoxic effects[12],[13]. Further, η-secretase directed alternative processing route is also reported under physiological conditions. It is also observed that neurotoxic amyloid plaques recruited Aβ is involved in the formation of insoluble aggregates which provokes impaired mitochondrial dynamics in the hippocampus and basal segment. It also hampers normal synaptic functions and leads to impair brain homeostasis [14] [15]. It is also evident that Ab induced microglia and astrocytes induce neuroinflammatory reactions and oxidation, which causes AD associated neuronal dysfunction and apoptosis. Ab

induced Tau protein kinase 1 can cause abnormal tau phosphorylation and promote the production of NFTs and pair helical filaments (PHFs), which enhance tau pathology [16]. Further "Aβ oligomer pathogenic theory," officially proposed by Ferreira in 2011 also advocates that the initiating factors causes pathological changes in AD is Aβ oligomers [17]. Elevated level of Aβ oligomers is reported as evident in many studies [18]. Accumulation of Aβ oligomers occurs by acting on NMDA-α7-nACh receptors contribute to enhanced LTD as well as LTP inhibition [19][20]. This further leads to altered synaptic functions, learning and memory impairment [21]. Neurotoxic Aβ42 oligomers have also potential to hamper synaptic membranes induced by oxidative stress and provoke tau protein hyperphosphorylation [22][23]. Considering the pathological hallmarks of AD, it is also imperative to find proper remedies for its pathogenesis. Hence, the main aim of Aβ hypothesis based therapeutic strategies are to curtail Aβ accumulation and its formation and enhanced Aβ clearance [24]. In order to reduce Aβ production the most direct action is BACE1 and γ-secretase modulation [25][26]. Although, γ-secretase inhibitors are toxic to many organs as lack substrate specificity for APP [27].

Furthermore, considering clinical trials, the AD drug avagacestat, shown severe serious side effects including rashes, GI reactions and also triggers tumors progression, and did not show any desired results; and hence clinical trials have terminated [28]. Similarly, Encore's tarenflurbil usage, was likewise ceased due to no positive impact in cognitive impairment, however which has good security. The use of other drugs such as semagacestat have been terminated owing to its increased adverse reactions. The use of semagacestat was unsatisfactory until now, as occasionally it outperformed placebos. Considering core areas for anti-AD drug development, BACE1 inhibitors may have potential as they possess

higher substrate specificity. Moreover, it also reportedly shown unanticipated adverse side effects hence, not shown significant clinical benefits[29] as evident in many Phase III trials, although it can curtail Aβ levels in CSF by up to 90%. The clinical trials were announced to terminate early in 2018. In 2019 July, due to cognitive deterioration in participants, Phase II/III investigation of the umibecestat a well-known BACE1 inhibitor was also ceased and terminated. Additionally, animal experiments suggested that it has potential to accelerate the clearance and Aβ degradation [30]. It was also reported to promote periphery Aβ delivery [31]. Further to manage AD, active and passive immunity are one of the major research hot spots as far as immunotherapy is concerned. For instance, crenezumab and aducanumab, were terminated in some Phase III investigation, owing to ineffectiveness and inability to reach the primary target, however they had the capability to curtail Aβ deposition. Very recently, scientists by using endo glycosylation receptor inhibitor TTP488 (azeliragon) strived to curtail aggregation of Aβ to ameliorate neuropathology and cognition in animal model[32]. It also showed significant cognitive improvement in Phase II trials[33]. However, lacking of desired effects, the clinical Phase III trial of azeliragon was terminated. Similarly, small synthetic alpha-peptides developed by researchers at the University of Washington. These peptides target and impede deleterious oligomers formation and prevent Aβ accumulation at initial stage. In AD *Caenorhabditis elegans* mouse model good results were observed however, its efficacy is yet to be studied in humans[34].

2.1.2 Tau hypothesis

The MAPT gene's alternative splicing results in the production of tau[34]. The first evidence of tau that may be one of the contributory factors of dementia was presented in 1988 by Claude Wischik after extraction of tau protein from the neurotic plaques[35]. Tau reside primarily in microtubules and axons[36]. Tau has a decisive part in the maintainence

of the function and structure of synapse and microtubule [37][38]. Tau is another phosphoprotein whose dephosphorylation and phosphorylation activity may be influenced by the equilibrium of protein phosphatase and kinase activities. Tau has some phosphorylation sites in its native form that negatively control its binding to microtubules while under pathogenic circumstances, tau's phosphorylation became excessive. Tau pathology is complicated and depends upon multiple factors. In the AD brains, hyperphosphorylated tau alters tubulin's structure and impairs its ability to polymerize, impairing microtubule activity[39].The development of insoluble NFTs, is facilitated by the increased amounts of cytoplasmic tau[40]. NFTs disrupt cell function, synapses and are neurotoxic[41] Studies have exhibited a favourable link up between tau hyperphosphorylation and the clinical severity of AD [41]. Cognitive state is determined by tau in lieu of Aβ [42]. Furthermore, tau's capacity to adhere to microtubules is inhibited by truncation and acetylation, which additionally fosters synaptic impairments, tau accumulation, and mitochondrial malfunction[43][44][45]. P-tau possesses the interesting ability to migrate across cells [45]. Tau could be more detrimental than NFT, which can influence cognition and neurodegeneration in addition to aiding the spread of pathogenic tau [46]. In conclusion, considering its intricacy, the pathophysiology of pathogenic tau is still not completely comprehended. Tau has drawn really close focus lately, in part because several Aβ targeting therapies have failed in clinical trials. Recent research has been concentrated on microtubule stabilizers, immunotherapeutic medicines, and kinase and aggregated tau inhibitors[47].

2.1.3 Cholinergic Hypothesis

Acetylcholine (ACh) is synthesized by the enzyme choline acetyltransferase (ChAT), which has been linked to presynaptic and neocortical cholinergic deficiencies since the 1970s. ACh has major roles in cognition, hence a cholinergic hypothesis of AD emerged

to the limelight. The ACh is produced upon action of ChAT enzyme in the cholinergic neuron's cytoplasm from acetyl-coenzyme A and choline. The VAChT transfers it to the synaptic vesicles for further processing. ACh plays vital role in memory functioning, learning, and sensations and other crucial activities of the brain are also controlled by its presence. Any impairment in cholinergic neurons results in loss of cognitive capacities and memory and hence thought to be associated with AD progression. Neurotoxic Aβ is supposed to obstruct with cholinergic neural communication, curtails choline assimilation, and stimulate Ach production. The neurotoxicity of Aβ and interconnection between AChE and Aβ have been reported to associate with cholinergic synaptic impairment and the amyloid fibrils formation. The loss of muscarinic (M2) and nicotinic Ach receptors, present on presynapse of cholinergic neurons, and loss in EAA neuronal communication, where in D-aspartate absorption and glutamate level are drastically decreased in cortex of AD brains. Additionally, scopolamine (cholinergic receptor antagonist), that has demonstrated to develop amnesia, are utilized. The impact could be mitigated by utilizing agents that increase acetylcholine production [48][49].Therefore, the cholinergic hypothesis relies on the following three concepts: the significance of cholinergic inhibitors in cognitive decline as in contrast to agonists, with a reverse impact, decreased levels of cholinergic markers in the cortex, severe neurological damage in NBM [50].

2.2 Wnt cascade

The Wnt/β-catenin cascade regulates major functions of organisms including cell migration, differentiation, proliferation and is highly conserved among the metazoans throughout the evolution [30]. This pathway plays a critical part in the advancement of CNS and is involved in the functional regulation of the adult brain, specifically in the regulation of the function of mature neurons [51] [52]. This developmental signaling is also

associated with other regulatory functions of the brain such as neuronal migration, neural maturation, axonal differentiation, synapse formation and neural connectivity [53] [54] Additionally, in the mature brain, this pathway regulates neural plasticity and synapse functioning. Mammals possess Wnt proteins encoded by 19 Wnt genes which are classified into 12 sub families that are quintessentially glycosylated (lipid-modified) cysteine-rich proteins having molecular weight in the range of 39-46 kDa [55] [56]. Wnt ligand interaction with the transmembrane frizzled (Fz) receptor marks the initiation of the canonical Wnt cascade. While, the non-canonical Wnt cascade activation is triggered by Wnt ligands and LRP5/6 interaction. After interaction between ligand and receptor, the signaling activation is stimulated by the Dishevelled (Dvl) protein binding which further mobilizes the degradation complex. The degradation complex consist transcription factors/proteins such as Axin, glycogen synthase kinase-3 (GSK-3), protein phosphatase 2A (PP2A), casein kinase 1α (CK1α) and adenomatous polyposis coli (APC).The mobilization of the degradation complex allows the aggregation of β-catenin in the cytoplasm which subsequently, translocate into the nucleus, binds with TCF/LEF transcription factors and initiate transcriptional activation of Wnt target genes[57].Thereby, upregulation of downregulated Wnt signaling can be a promising therapeutic target to mitigate AD pathogenesis.

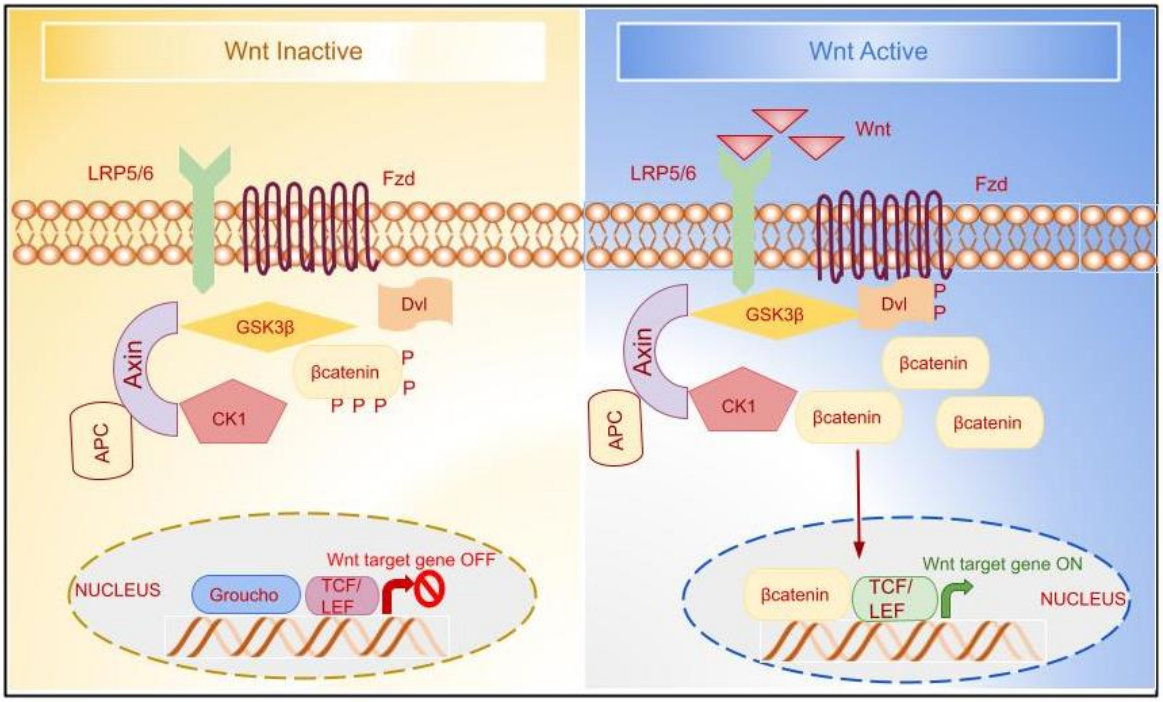

Figure2.1: Canonical Wnt cascade the figure represents the Wnt/β-catenin pathway in transcriptionally /OFF (left side) and ON (right side) states. In absence of Wnt, Fzd(frizzled) and LRP5/6 are unable to associate and hence β-catenin gets phosphorylated by GSK-3β in the complex, tagging it for degradation which leads to turning off of Wnt genes. Whereas in active (ON) state, the degradation complex gets attached to the receptors in the membrane which increases intracellular β-catenin levels which then turns transcription of Wnt genes ON.

2.2.1. A neurological association of Wnt signaling in Alzheimer's disease

AD is the most prevalent neurodegenerative condition with risk factors including increasing age and oxidative stress [58][59]. Several signaling cascades are allied with the pathophysiology of AD and Wnt/β-catenin cascade is one of them. Any alteration in this signaling cascade of the aged brain can lead to curtail cognitive deficits and neurogenes [60]. In the aged brain, low expression of various disheveled (Dvl) proteins (such as Dvl2 and Dvl3) and Wnt proteins including Wnt 2, 3, 4, Wnt7b and Wnt10b has observed to be reduced, whereas DKK1 activity (Wnt antagonist) found to be enhanced, ultimately causing the downregulation of Wnt/β-catenin cascade[61][62][63][64][56][65][66][67]. Notably, adult neurogenesis is altered by age related low levels of Wnt proteins in astrocytes [64][68], while exercise-assisted restoration of Wnt protein levels augments neurogenesis

in adults[69]. In AD brain LRP6 alteration resulted in reduced Wnt/β-catenin level. An alternatively splice variant and two LRP6 SNPs are reportedly linked to increased tendency of AD progression associated with altered Wnt/β-catenin cascade[70][71]. The LRP6 expression is reduced in the AD brain, and reduced LRP6-mediated Wnt/β-catenin cascade can lead to amyloid pathology and synaptic impairment in AD [72]. In neuronal PC-12 cells expressing LRP6 apolipoprotein E4 (ApoE4) is known to suppress Wnt/β-catenin activation [73][74]. LRP6 suppresses Aβ production by physically interacting with APP [70][75], whereas reduced Wnt/β-catenin activation has been displayed by the Swedish familial AD variant of APP (APPSwe)[75]. Further, toxic Aβ peptides can inhibit Wnt/β-catenin signaling by inducing DKK1 expression in primary cortical neurons, and DKK1 expression can induce synapse degeneration in the adult hippocampus [76][77]. The inhibition of GSK3β, consequently trigger Wnt/β-catenin cascade activation by interaction of Wnt protein to Fzd/LRP[78]. Higher expression of GSK3β kinase is accountable for the phosphorylation and degradation of β-catenin [79]. Further in the AD brain, down-regulation of LRP6 and up-regulation of DKK1 resulted in higher expression of GSK3β [80][81]. Lately, it has been observed that GSK3β higher activation is inversely correlated with a considerable reduction in β-catenin levels in AD brain[82]. This study further strengthens the idea that GSK3β activation and Wnt/β-catenin cascade is interlinked with each other in the AD brain. Interestingly, GSK3β is the prime kinase accountable for tau phosphorylation, and GSK3β overactivation is associated with tau hyperphosphorylation, Aβ agglomeration, and memory dysfunction [83][84][85]. It has also been observed in recent studies, where primary neurons showed β-catenin degradation was promoted by overexpression of APP and its mutants, while opposite effects were observed after APP knockdown [86].

2.2.2 Interconnection between T2DM, AD and Wnt Signaling

Beta cells in pancreas secrete insulin that plays a vital part in regulating blood glucose level. Although insulin receptors are present on the skeletal, hepatic and adipose tissues, it has also been reported that they are also present in nervous tissue majorly in the region accountable for cognition, hunger and involuntary activities[87][88][89]. Intriguingly, it has been observed that insulin might not only be produced by beta cells of pancreas but may also be produced *de novo* in the glial cells of the CNS [88][90]. Nonetheless, irrespective of the site of generation, the mode of action of insulin remains the same in the CNS and periphery as well [91] [92]. Insulin resistance is referred as impaired response of host tissue to insulin hormone. It plays a significant part in T2DM pathophysiology[93]. The main cause of insulin resistance is the inability of insulin receptors to bind with insulin and thereby downregulating the downstream signaling mediators[94]. Moreover, impaired brain insulin sensitivity can result in altered neurotransmission, brain plasticity, and neuronal growth[95].Initiation of insulin signaling is marked by the interaction of insulin with IR which further activates IRS-1, and subsequent activation of PI3K and Akt, and finally activation of target genes including GSK3β, FOXO, and mTOR respectively[96][97][98]. It has been observed that insulin resistance is mediated by ceramide induced IRS-1 phosphorylation, resulting in the prohibition of PI3K/Akt signaling in CNS. Intriguingly, ceramide accumulation stimulates a number of serine-threonine kinases that phosphorylate serine and prohibit IRS-1, further leading to the condition termed as brain insulin resistance. Subsequently, ceramide accumulation and permeation through BBB not only dysregulates downregulates the Akt signaling but also provoke interleukin-1β converting enzyme (ICE) that further result in the impaired insulin signaling cascade and neuronal death[99]. Lately, several studies have linked insulin resistance and neurodegeneration[91] these aberrations can be elucidated by chronic

inflammation, dyslipidemia, hyperinsulinemia, hyperglycemia, mitochondrial dysfunctions and free radical generation in brain [100][101].AD is manifested by the loss of memory and cognitive dysfunction due to tau hyperphosphorylation and Aβ agglomeration [67][68]. Even though the exact pathophysiology is ambiguous, several studies have suggested altered insulin signaling associated with AD brain, thereby referring to AD as “type 3 diabetes” or “brain diabetes”[87][102][103][104]. Nonetheless, disturbed cerebral glucose uptake and impaired insulin sensitivity have been observed in the EOAD. De la Monte et al. reported reduced expression of IRS-1, PI3K/Akt, IGF-1 and 2 and insulin receptor and elevated level of GSK-3β in AD brain. Further, it is also evident that brain IR leads to the enhanced activation of GSK-3β in AD brain [105][106] whose stimulation fosters the tau hyperphosphorylation and thereby causing NFTs formation[107]. Emerging research recently demonstrated that any impairment in Wnt signaling cascade has crucial impact on the pathogenesis of T2DM by directly affecting insulin production and action, pancreatic-cell differentiation and proliferation. However, given their various components and intricate networks, Wnt pathways' role in the pathophysiology of T2DM and its consequences looks counterintuitive. The preliminary investigation into the association between T2DM and Wnt signaling pathways was published in 2006 by Grant et al. By controlling the activity of proglucagon gene, researchers discovered that genetic variation of the TCFL2 gene, which expresses the crucial transcription factor TCF4 in Wnt signaling pathways, increased the risk of T2DM and 85% of the amino acid sequences of Wnt3 and Wnt3a are found to be commensurate. Studies on Wnt3 currently are mostly concerned with its role in diabetic complications of the CNS rather than diabetes. According to a recent study, the Wnt3/-catenin pathway was inhibited in diabetic rats, which resulted in reduced cognitive performance and a loss of neurogenesis. Insulin treatment enhanced Wnt3 expression in astrocytes and thereby boosted neurogenesis. Likewise, treadmill exercise

attenuated AD induced cognitive impairment and boosted neurogenesis in diabetic rats via activating the prohibited canonical Wnt cascade. Resveratrol administration discovered to boost the production of Wnt7a and proliferation in the hippocampus diabetic rats, suggesting restorative function of Wnt7a in diabetes. In a study, novel and significant link between Wnt and insulin signaling cascade unveils the impact of metformin on the synthesis of GLP1. Further, Drugs of the α-glucosidase inhibitor class and PPAR agonists were discovered having substantial affinity for LRP6, emphasizing the possibility that Wnt signaling may be modulated by antidiabetic drugs used for AD. Moreover, Aβ peptides compete with the insulin and reduces the ability of insulin to bind to insulin receptor and thereby hampers insulin transmission in the AD brain[108]. Further, down regulating the PI3K/Akt signaling and enhancing GSK-3β activity, thereby downregulation the Wnt signaling pathway and hence AD pathogenesis[108].Lately, a number of studies have revealed promising evidence for the connection between AD and diabetes. AD is contemplated as brain-specific diabetes and the term ‘Type 3 diabetes was proposed by De la Monte for insulin resistance mediated AD. Impaired insulin signaling and insulin resistance in the CNS has dynamically established a strong connection between AD and type 2 diabetes

2.2.3 Susceptibility of diabetes : Implication of Wnt pathway Components

Research studies reported that the Wnt signaling has a crucial role in regulating glucose and lipid metabolism and thereby the etiology of metabolic diseases. LRP-5 and LRP-6 are two important co-receptors for the Wnt ligands [109]. Polymorphism in LRP5 has been strongly connected to obesity [110]. After being provided with a high-fat diet (HFD), LRP-

5 knockout mice (homozygous) exhibited elevated levels of plasma cholesterol [111]. The LRP-5/ mice also the LRP-5/ mice demonstrated noticeably decreased glucose tolerance when fed a regular diet[111]. Moreover, when subjected to high glucose interventions, LRP-5/ animals showed a substantial drop in calcium and ATP levels in tandem with a reduction in glucose-induced insulin secretion. Eventually , Wnt 5a and Wnt 3a were capable of inducing insulin secretion [112] in wild-type in contrast to in LRP-5(-/-) mouse[112]. Therefore Wnt-5a and Wnt-3a are contingent upon LRP-5's to promote the secretion of insulin. Kanazawa and associates investigated the connection between T2D and Wnt family genes in the Japanese community [113].They examined 40 SNPs in 11 Wnt genes analyzed, and it was found that six of those examined showed substantial variations in the allele between T2D and controls. Interestingly, an SNP in Wnt-5b, had been closely linked to T2D. Wnt-5b seems to be a suppressive Wnt ligand, with the capability to prohibit Wnt activation [113],[114]. Additionally, Wnt-5b and Wnt-5a conjointly function to control differentiation and growth of chondrocyte[114].Therefore Wnt cascade has a pivotal role in onset of diabetes.

2.2.4 Controversial observation on the role of TCFL2 in pancreatic beta cells

Several studies on TCF7L2's function in pancreatic-cells hinted at potential negative implications. For instance, Lyssenko et al. revealed that , in SNP rs7903146 the CT/TT genotype is closely connected with an increased likelihood of T2D in two distinct cohorts [115].They noted that TCF7L2 mRNA levels in the pancreatic islets of T2D patients were elevated. Additionally, the pancreatic islets of T allele carrier demonstrated a significant rise in TCF7L2 mRNA expression, which was linked to reduced production of insulin and incretin levels[115]. Another transgenic mice research likewise demonstrated the TCF7L2 detrimental impact on islets of the pancreas [116]. These outcomes, nonetheless, are contrary to research by Shu and colleagues, which suggested that TCF7L2 may have

positive impacts on pancreatic beta cells [117][118].They discovered that siRNA-mediated TCF7L2 deletion significantly increased beta cell death and decreased the proliferation of beta cells in isolated human or mouse pancreatic islets, which were connected with an impairment in the glucose-stimulated secretion of insulin[118]. On the contrary, higher expression of TCFL2 preserved islets against cytokine and glucose induced cell death[118]. Another study found that TCF7L2 protein levels were actually lowered, despite higher TCF7L2 mRNA levels in the islets of T2D models[117]. Simultaneously, the activity of GLP-1R, GIPR ,and TCF7L2 was likewise downregulated in human islets as well as in isolated islets attenuated with TCF7L2 using siRNA therapy[117]. The expression of various TCF7L2 isoforms in specific cell type might also be responsible for some divergent opinions by various studies regarding the advantageous versus detrimental effect of TCF7L2 in pancreatic beta-cells[117][118][119].

CHAPTER-3

METHODOLOGY

3.1 Data Collection

A list of 143 FDA approved antidiabetic drugs was compiled from published literature [120]. These underwent evaluation for their capability to permeate BBB using the online predictor Light BBB (https://github.com/12rajnish/DeePredBBB) .114 out of 143 have been determined to be BBB permeable. Five drugs were selected with docking score less than control. These were downloaded from Pubchem in .3d sdf format and then converted to .mol2 format using an online portal "Convert" (https://datascience.unm.edu/tomcat/biocomp/convert).The target molecule for this study is Glycogen synthase kinase 3 beta.

Table I: Antidiabetic medicines with docking score against GSK 3 beta

S.No	Compound	PubChem Id	Chemical Formula	Binding Energy (Kcal/mol)
1	Metformin	4091	$C_4H_{11}N_5$	-17.24
2	Nateglinide	5311309	$C_{19}H_{27}NO_3$	-11.72
3	Glimepiride	3476	$C_{24}H_{34}N_4O_5S$	-11.34

4	Glipizide	3478	$C_{21}H_{27}N_5O_4S$	-10.1
5	Rosiglitazone	5281055	$C_{22}H_{23}N_3O_7S$	-10.01

3.2 Protein Preparation

The PDB crystal 1J1C (GSK 3β) has 2.10 Å resolution and comprises three domains: a C-terminal domain consisting of a 'kinase fold', an N-terminal domain comprising of a beta barrel structure and an extra domain consecutive to C-terminal. Steps like addition of hydrogen atoms and removal of crystallized water molecules were performed in order to prepare the protein for molecular docking using the BIOVIA discovery studio. The protein molecule (prepared) was then saved in PDB format.

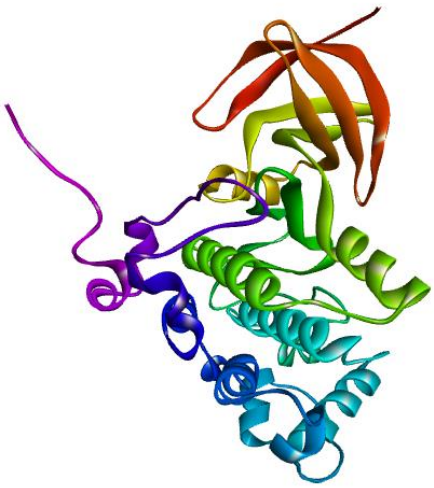

Figure3.1: PDB Structure of GSK 3 β (1J1C)

3.3Pharmacophore modelling

The fundamental geometric configuration of atoms or functional groups required to elicit a specific biological reaction is referred to as a pharmacophore. Potential leads for the discovery of drugs closely resemble a defined pharmacophore. Pharmacophore modeling was done using an online webserver ZincPharmer (http://zincpharmer.csb.pitt.edu/). It is an online platform for scanning chemical compounds that can be purchased from the ZINC database. The drugs were uploaded in the ZincPharmer server in .mol2 format. The top hits along with their ZINC ids are depicted in the Table.

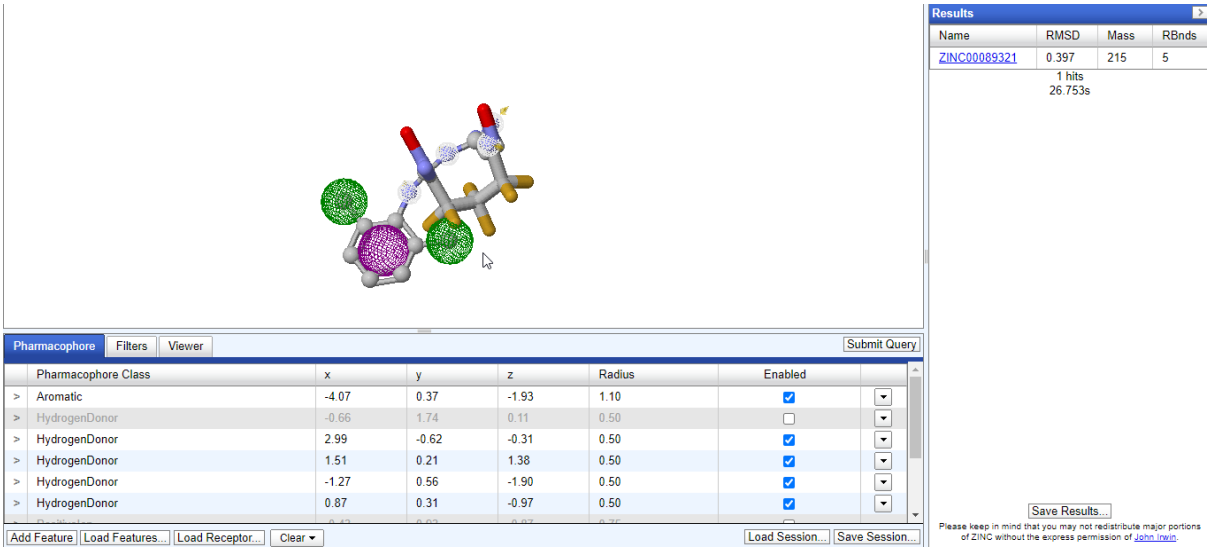

Figure3.2: Interface of ZINCPharmer

3.4 Molecular Docking

It is a computational method to spiculate the ideal orientation of a ligand when it binds with a target. It is an efficient technique of investigating the strength interactions between ligand and target. Molecular docking was performed using a web service

Swiss Dock **(**http://www.swissdock.ch/). Ultimately the compounds were positioned on the basis of the docking scores and ligands with binding energy less than already known inhibitors were considered.

3.5 ADMET Analysis

The drugs were put through ADMET analysis using Swiss ADME (http://www.swissadme.ch). Properties like blood-brain barrier (BBB) permeability, carcinogenicity, half-life, log p value were analyzed.

3.6 MD Simulation

The stability of binding between docked complexes is determined by MD simulation. The MD simulation additionally provides details on interactions between molecules taking place within a reference timeframe. In this study this procedure was carried out on the best docked complex using GROMACS for the interval of 75 ns.

CHAPTER-4

RESULT

Molecular Docking Analaysis

ZINC04803471, ZINC01706102, ZINC05604231, ZINC94246440 had the less binding energies than the already approved control which are represented in the Table II.

Figure4.1: ZINC04803471 binding to GSK 3 beta

Figure4.2: Binding of ZINC01706102 to GSK 3 beta

Figure4.3: Binding of ZINC94246440 to GSK 3 beta

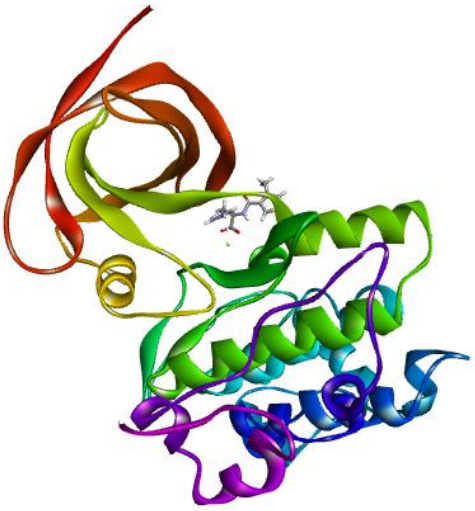

Figure4.4: Binding of ZINC05604231 to GSK 3 beta

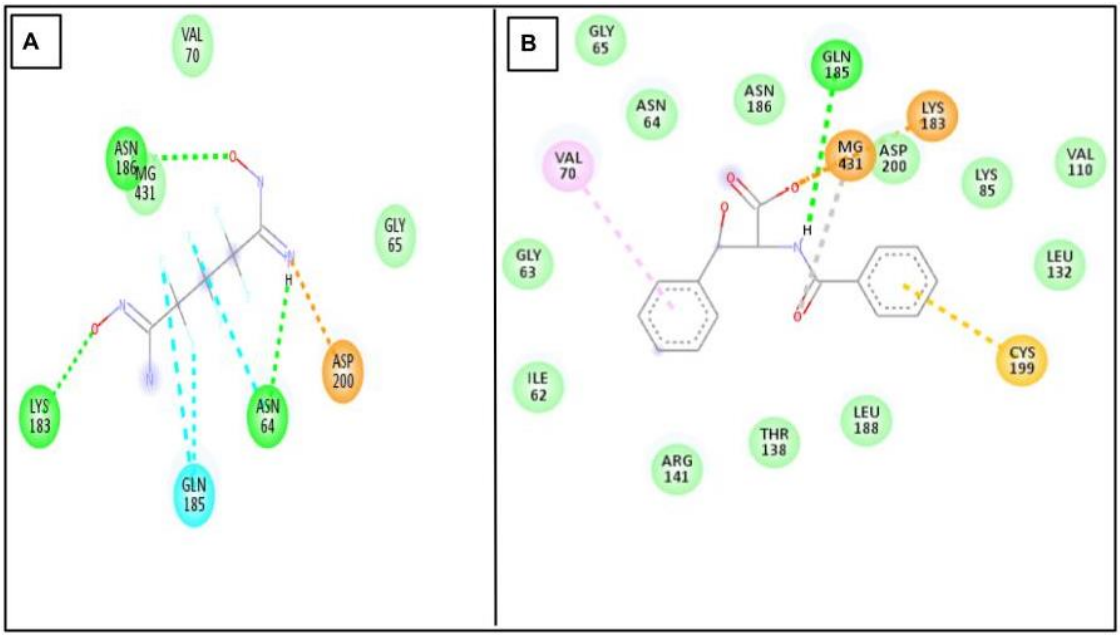

Figure4.5: 2D structures of ZINC04803471, ZINC01706102

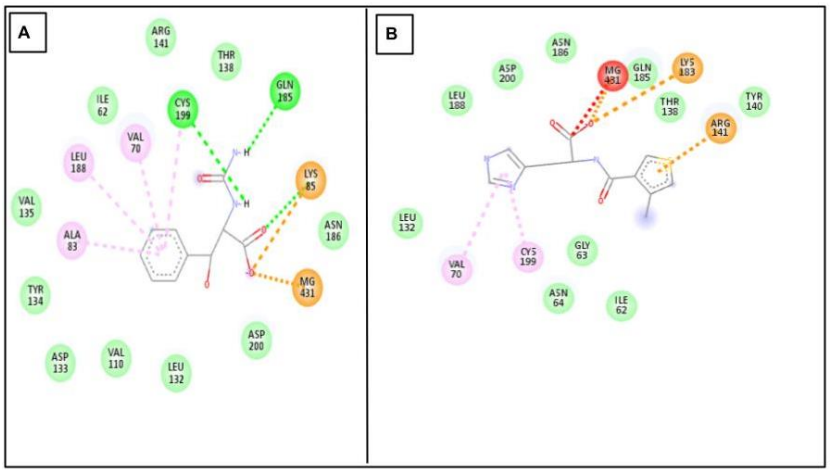

Figure4.6: 2D structures of ZINC05604231, ZINC94246440

Table II: Table depicting compounds with binding energies less than control

S.No	Compound	Docking Score (Kcal/mol)
1	ZINC04803471	-17.58
2	ZINC01706102	-11.26
3	ZINC05604231	-10.4
4	ZINC94246440	-10.15

MD Simulation Analysis

RMSD Analysis

The average distance that results from an atom's displacement for a particular period of time relative to a reference time frame is measured using the RMSD in MD simulations. After computing the RMSD of the protein fitted ligand across all the time intervals throughout the reference time (75 ns), the RMSD of a specific protein structure, such as the C-α is determined.

It was observed that the initial RMSD was at 0.2 nm which increased to 0.4 nm at the first 13 ns. However, the RMSD was stable with 0.2 ns from 15 ns to 40 ns. Post 40 ns, a sudden rise in RMSD was observed which settle down to 0.2 nm at the 74 ns simulation trajectory. Few fluctuations were observed which indicated the conformation change of the protein upon binding to ligand. RMSD plot suggested that the protein acquired stable conformation after the 74 ns simulation trajectory.

RMSD of Ligand

RMSD plot of the ligand bound to the protein showed stable and consistent RMSD. It was observed that the initial RMSD was at 0.2 nm which increased to 0.7 nm at the first 5 ns. However, the RMSD was consistent with 0.7 nm for the rest of the 75 ns simulation. Few fluctuations in the RMSD were observed. However once the ligand was bound at a stable binding conformation with the protein, it became stable.

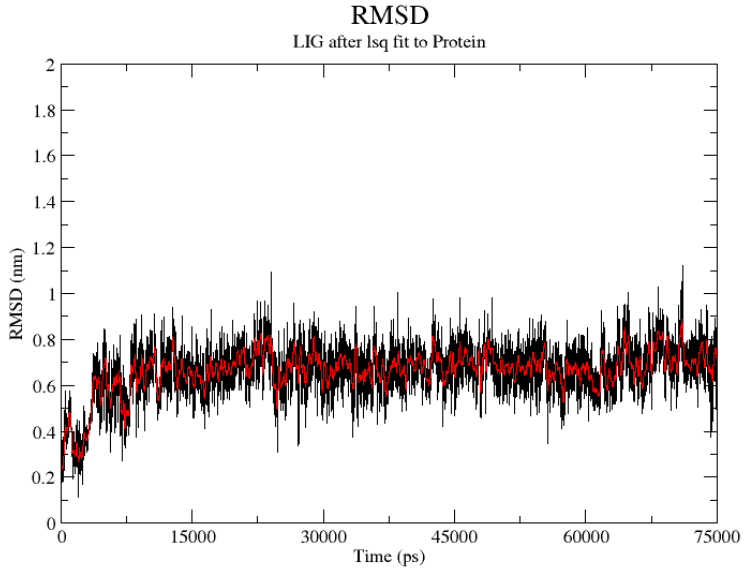

Figure 4.7. RMSD for ligand aligned over the initial structure of protein.

RMSD of Protein

It was observed that the initial RMSD was at 0.2 nm which increased to 0.4 nm at the first 13 ns. However, the RMSD was stable with 0.2 ns from 15 ns to 40 ns.Post 40 ns, a sudden rise in RMSD was observed which settle down to 0.2 nm at the 74 ns simulation trajectory. Few fluctuations were observed which indicated the conformation change of the protein upon binding to ligand. RMSD plot suggested that the protein acquired stable conformation after the 74 ns simulation trajectory.

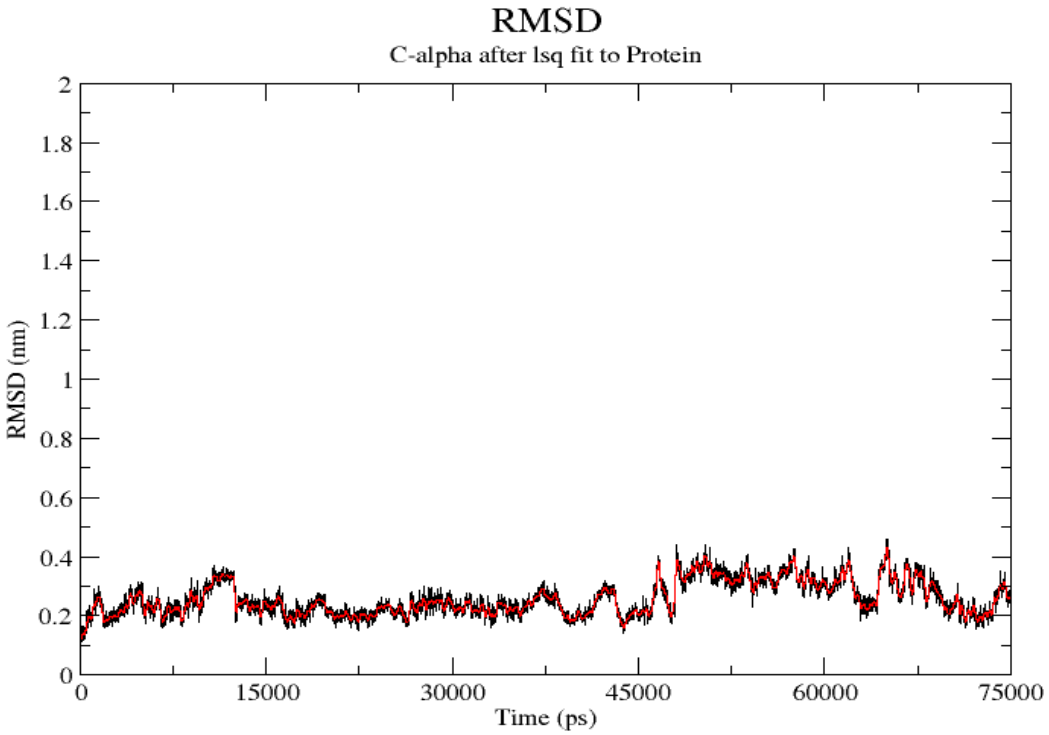

Figure4.8 RMSD of protein Cα atoms where initial equilibrated structure was considered as the reference for calculating the RMSD.

RMSF Analysis

RMSF plot of the protein upon binding to the protein had been shown here. Majority of the residues of the protein showed RMSF < 0.3 nm. Few fluctuations were observed during the 75 ns of the simulation.

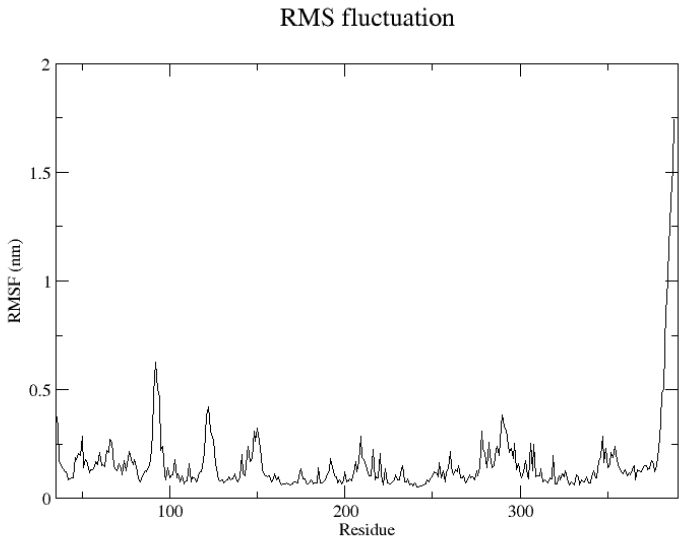

Figure 4.9. RMSF of protein residues bound with ligand.

CHAPTER-5

CONCLUSION

Therapies of AD have not been formulated till date. There are some drugs in the market but they only provide symptomatic relief. Therefore, in recent years drug repurposing using *in silico* techniques such as molecular docking has helped to facilitate the drug development procedure. Using this technique, we identified ZINC04803471 compound as potent inhibitor of GSK-3β which is a mediator molecule in Wnt signaling pathway. It has shown promising effect as a better inhibitor with binding energy -17.58 in comparison to FDA approved inhibitor indirubin-3-oxime with binding energy of -9.07. Three other compounds were also found to have less binding energy than the inhibitor, these compounds are ZINC01706102, ZINC05604231, ZINC94246440. This inhibition is quite favourable in restoration of downregulated Wnt signaling in AD brain as GSK 3 beta will be inhibited as a result there will not be beta catenin degradation, which further leads to translocation of beta catenin to nucleus and eventually activation of TCF/LEF. However wet lab investigations need to be done for further validation.

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LIST OF PUBLICATIONS

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Targeting GSK-3β for the Modulation of Wnt Signaling Pathway in Alzheimer's Disease: A Drug Repurposing Approach.

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***Abstract*- Alzheimer's Disease is insidious, progressive disorder whose pathophysiology and therapeutic status still stands unclear. As of now, all the therapies are confined to symptomatic relief, disease modifying therapies are therefore the needof the hour. Modulation in Wnt cascade has already beenlinked to varied disorders which include AD and type 2 diabetes mellitus too. The cross-talk between insulin signaling pathway and Wnt signaling has been well acknowledged through a number of preclinical and clinical studies. This *in silico*-basedstudy is focused upon investigating the link between curative effects of FDA approved anti- diabetic drug and Wnt signaling cascade. We prepared library consisting of 143 FDA approved antidiabetic medicines with an aim of repurposingthem as GSK 3 beta inhibitor, which is present in increased amounts in AD brain. Through ligand- protein based molecular docking we identified metformin, an antidiabetic drug that can inhibit GSK 3 beta, which leads to beta catenin degeneration and thereby downregulates the canonical Wnt signaling cascade in AD brain.**

Keywords: GSK 3β, Alzheimer's disease, Wnt signaling cascade, drug repurposing.

I. INTRODUCTION

Wnt signaling pathway is a signal transduction pathway that regulates cell proliferation, cell migration, tissue homeostasis, synaptic plasticity, and aging [1]. Wnt also plays a significant role in synaptogenesis, neural patterning, and neuronal polarity throughout the development of embryonic Central Nervous System. Mammals have 19 Wnt genes which are grouped into 12 subfamilies. These ligands bind to the heterodimeric receptor complex present on the surface of the cell constituting co-receptors LRP6/5 and Frizzled (Fz) receptor as well. 7-transmembrane (7TM) receptors are encoded by 10 Fz genes possessed by mammals. Wnt ligands initiate the signaling pathway upon interaction with the cell surface receptors, which further leads to conformational changes. The binding of the Fz-LRP receptor complex to the Wnt ligand marks the initiation of the Wnt/β catenin cascade, which further cause degradation complex deactivation comprising APC, Axin, GSK-3β, and CK1α, which is responsible for phosphorylating β- catenin, thereby symbolizing its proteasomal degradation. β-catenin translocate into nucleus where it's interaction with the TCF/LEF leads to initiation of the Wnt/β-catenin target genes transcription which have a known role in cell differentiation, cell fate specification, cell proliferation, and tissue homeostasis (Figure1).

AD is progressive neurodegenerative ailment marked by cognitive and memory loss majorly targeting the aged population [2]. It has been ranked sixth among the leading causes of death around the globe. However, the exact pathophysiology stands unclear, aggregation of neurofibrillary tangles and beta amyloid plaque are the pathological hallmarks of Alzheimer's Disease [3]. Furthermore, over the period of time AD has been associated with oxidative damage, insulin resistance, mitochondrial dysfunction, neuroinflammation and amyloid hypothesis [4].

Subsequently, Wnt signaling Cascade also has a pivotal role in AD pathology, it is perhaps observed that the Wnt/ β catenin cascade is dysregulated/ suppressed in AD, which further hints at the potent link between AD and its major risk quotient- age. Most AD cases are diagnosed at later stages or later age (LOAD) validating the fact that AD is primarily an age-related disease of the brain. Moreover, mutations in some specific genes may lead to early-onset/ familial AD (FAD). Gene variants have also been linked to AD several times and genetic studies have played an invaluable role in exploring pathogenesis in AD. A study reported polymorphism in the GSK3β promoter, which resulted in the increase of GSK3β activity and thus increased the susceptivity to LOAD,given that increased activity of GSK-3β leads to decreased Wnt signaling, furthermore, promoting degradation of β-catenin. Several In vitro and In vivo researches have reported dysregulation of the Wnt/ β catenin cascade led to increased AD pathogenesis [5]. Restoration of dysregulated Wnt signaling may be foreseen as the potential therapeutic intervention. For instance, small molecules have been formulated to upregulate the Wnt/ β catenin cascade that eventually decreases neuronal loss[6]. As of now, there are only four FDA-approved medicines from just two types (acetylcholine inhibitor and NMDA antagonist) available for treating AD. But these medicines can only offer symptomatic alleviation. There is currently no disease-modification therapy for AD. Lately the focus has been shifted to repurposing of drugs, in order to find new therapeutic applications of an already available drug. Several of research studies has been conducted to analyze the curative potential of antidiabetic drugs for treating AD.Different classes of antidiabetic drugs have shown promising results in reviving impaired memory, alleviating neuroinflammation and lowering oxidative damage [7]. In this study, we have used in silico toolsto analyze the curative potential of antidiabetic drugs on dysregulated Wnt signaling mediator GSK 3 beta for the possible treatment of AD. Herein we determined that metformin emerged as the powerful inhibitor to modulate Wnt signaling cascade in AD.

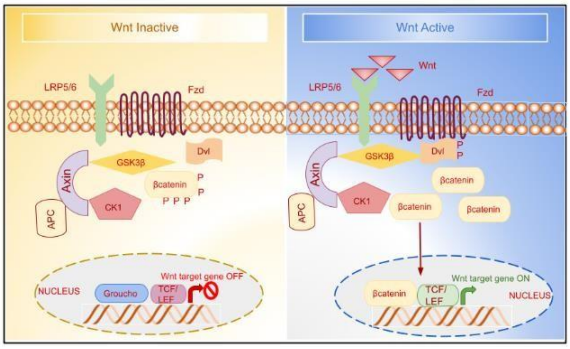

Fig. 1. The figure represents the Wnt/β-catenin pathway in transcriptionally /OFF (left side) and ON (right side) states. In absence of Wnt, Fzd (frizzled) and LRP5/6 are unable to associate and hence β-catenin gets phosphorylated by GSK-3β in the complex, tagging it for degradation which leads to turning off of Wnt genes. Whereas in active (ON) state, the degradation complex gets attached to the receptors in the membrane which increases intracellular β-catenin levels which then turns transcription of Wnt genes ON.

II. INTERCONNECTION BETWEEN TYPE 2DIABETES AND AD

Insulin resistance and persistent inflammation are twocharacteristics of type II diabetes mellitus (T2DM), a chronic illness associated with aging [8]. A number ofgenomic studies reported that the Wnt signalingcascade is connected to the majority of the genetic loci that are predisposed to the emergence of T2DM.TCFL2, an important member of the TCF/LEF family,is considered a novel risk gene for type 2 diabetes.Figeac et al. observed in neonatal diabetic rats thatelevated levels of GSK-3β led to the inhibition ofclassical Wnt signaling pathway. Wnt/β-cateninsignaling plays an important role in the production ofinsulin, and function of islet cells and it is observedthat Wnt signaling is downregulated in beta cells [9]. Lately, a number of studies have revealed promising evidence for the connection between AD and diabetes. AD is contemplated as brain-specific diabetes and theterm ‘Type 3 diabetes was proposed by De la Montefor insulin resistance mediated AD [10]. Impaired insulinsignaling and insulin resistance in the CNS has dynamically established a strong connection between AD and type 2 diabetes.

III. METHODOLOGY

All the in-silico studies based on the prediction of themolecular interactions were carried out using Swiss Dock software (http://www.swissdock.ch.) Windows operating system based 4 gigabyte random access memory system with i5 processor was operated for using tools such as BIOVIA discovery studio, Chimera.

A. Data Collection

A list of 143 antidiabetic medicines was prepared [11] [12]. These were subjected to screening for blood brain barrier penetrability using an online predictor Light BBB (https://github.com/12rajnish/DeePredBBB). Out of 143 drugs, 114 were found to be BBB permeable. Glycogen synthase kinase 3 beta was used as target molecule in this study (Figure2).

B. Ligand and Protein preparation

The list of antidiabetic drugs (FDA approved) was curated from published literature and drug bank. The PDB crystal 1J1C (GSK 3β) has 2.10 Åresolution and comprises three domains: a C-terminal domain consisting of a 'kinase fold', an N-terminal domain comprising of a beta barrel structure and an extra domain consecutive to C-terminal. Steps like addition of hydrogen atoms and removal of crystallized water molecule, were performed in order to prepare the protein for molecular docking using BIOVIA discovery studio. The protein molecule (prepared) was then saved in PDB format. The drugs were put through ADMET analysis using Swiss ADME (http://www.swissadme.ch). Properties like blood-brain barrier (BBB) permeability, carcinogenicity, half-life, log p value were analyzed. After ADMET analysis 37 molecules were selected for molecular docking analysis.

C. Molecular docking

Molecular docking is an efficient technique of predicting the interactions between ligand and protein. After protein and ligand preparation, molecular docking was performed using a web service Swiss Dock. Ultimately thecompounds were positioned on the basis of the docking scores and ligands with binding energy less than already known inhibitors were considered.

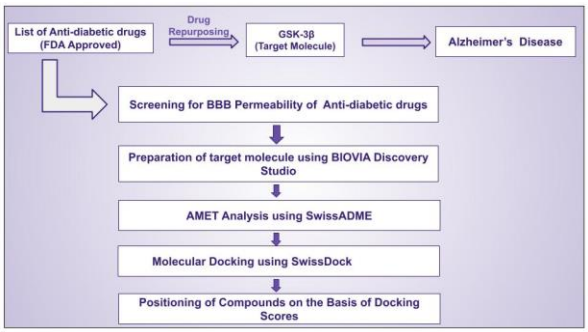

Fig. 2. Flowchart depicting methodology of the study.

IV. RESULTS

Only 1 out of 37 docked compounds met the requiredcriteria of binding energy less than the already knowninhibitor fostamatinib with binding energy of -15.62 (Figure 4).This drug which emerged as a better than known inhibitor is metformin with binding energy -17.24. Metformin is an antidiabetic drug of the class biguanide which is sold under the brand name Glucophage (Figure3). It has anti- aging propertiesas observed in several studies that metformin can delay the process of aging in mice. Ng et al observed in T2DM patients with metformin treatment for the period greater than six years had less risk of cognition loss [13]. The 2D visualization of interactions with both the drugs gives a better understanding (Figure5).

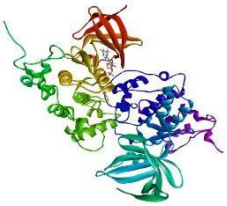

Fig . 3. Metformin binds to GSK-3β.

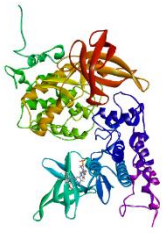

Fig . 4. Fostamatinib binds to GSK-3β.

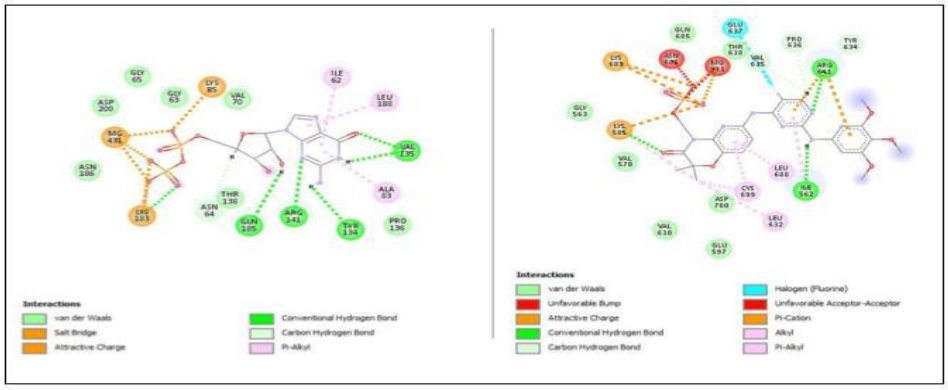

Fig. 5. 2D Structure visualization of interactions of Metformin (left) and fostamatinib (right).

V. DISCUSSION

Therapies of AD have not been formulated till date. There are some drugs in the market but they only provide symptomatic relief. Therefore, in the recent years drug repurposing using *in silico* techniques such as molecular docking has helped to facilitate the drug development procedure. Using this technique, we have repurposed an antidiabetic drug metformin as potent inhibitor of GSK-3β which is a mediator molecule in Wnt signaling pathway (Figure6). It has shown promising effectas a better inhibitor with binding energy -17.24 in comparison to other inhibitors such as fostamatinib with binding energy of -15.62 (Table1). This inhibition is quite favorable in restoration of downregulated Wnt signaling in AD brain as GSK 3 beta will be inhibited using metformin as a result there will not be beta catenin degradation, which further leads to nuclear translocation of beta-catenin and eventually transcriptional activation of TCF/LEF. A number of studies have been conducted that show the repercussion of metformin on tau and amyloid beta level. Furthermore, our study is also supported by other studies which reports metformin decreases tau phosphorylation in other diseases.[14] [15].

TABLE 1. DOCKING SCORE OF CONTROL AND COMPOUNDS AFTER PROTEIN LIGAND-BASED DOCKING WITH GLYCOGEN SYNTHASE KINASE BETA (COMPARISON WITH CONTROL).

COMPOUNDS	**DOCKING SCORE (kcal/mol)**
Metformin	-17.24
Nateglinide	-11.72
Glimepiride	-11.34
Glipizide	-10.10
Rosiglitazone maleate	-10.01
Gliclazide	-9.57
Acetohexamide	-9.55
Pioglitazone	-9.19
Rosiglitazone	-9.05
CONTROL	**DOCKING SCORE (kcal/mol)**
Fostamatinib	-15.62

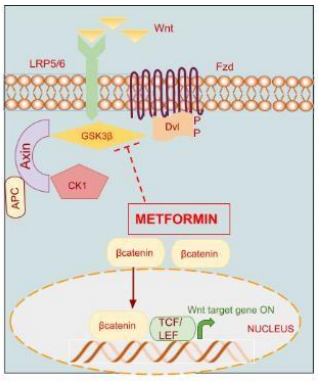

Fig. 6. The schematic represents the inhibition of GSK 3 beta by Metformin

VI. CONCLUSION

Currently the exact pathophysiology of the AD stands ambiguous. A boatload of studies has acknowledged the interlink between insulin signaling and Wnt signaling cascade. Moreover, the application of antidiabetic medicines in AD has emerged as promising therapeutic intervention for dementia and AD as well. We may infer from this *in silico* analysis that Metformin emerged as the powerful inhibitor with considerably less binding free energy in comparison to already known inhibitors. It has demonstrated excellent interaction with GSK-3β, thereby prohibiting beta-catenin degradation, eventually restoring the Wnt signaling cascade Therefore, this *in silico* model may emerge as potential therapeutic avenue for AD. Although *in silico* study is just a tip of an iceberg further In vivo and In vitro studies need to be done validate the proposed results.

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