2. Literature review

AD is a progressive neurodegenerative, irreversible fatal and a common disease in western world. Current knowledge hypothesize that Aβ pathology is the cause of all forms of AD. However, the hypothesis is consistent with very small portion of patients (< 5%) known to be FAD, and the cause may be due to mutations in APP and presenilin genes. Majority of cases are sporadic AD (SAD) are due to environmental factors including age, family history, cardiovascular risk factors, social engagement and diet. FDA has approved five drugs for the treatment of AD; merely none of them alter the underlying course of the disease. Number of treatment strategies is made available by the researchers throughout the globe that may have the potential to change the course of disease. One such strategy is DPP-4 inhibition/GLP-1 receptor activation. GLP-1 is an endogenous peptide synthesized and secreted from the L-cells of the gastrointestinal tract capable of crossing blood-brain barrier. It acts on GLP-1 receptors present in different brain regions including cortex, hippocampus and cerebellum to decrease Aβ plaques and APP.

2.1. Review on animal model used in the present study

Satiated literature available testifies the role of insulin receptors (IRs) in SAD. IRs are degraded predominantly in different regions especially hippocampus and prefrontal cortex in SAD. Loss of IR leads to abnormal brain glucose metabolism in SAD. IR degradation in T2D by a betacytotoxic STZ which enters through GLUT2 transporter into beta cell and induces DNA alkylation by a series of biomolecular cascade. The same mechanism is applicable when STZ is administered intracerebroventricularly (ICV) to induce SAD. Various models are available for preclinical investigation of a potential drug for the treatment of AD. Several transgenic models are being exercised to find novel therapeutics for AD. However, these transgenic
models represent AD induced by gene manipulation, unlikely representative to SAD. To surmount this challenge, STZ central administration is introduced to resemble human form of SAD. To convince this hypothesis, that STZ model induces features similar to human SAD following facts are considered: STZ causes abnormal glucose metabolism and utilization, loss of hippocampal volume and neuronal damage, and decline in cognitive and behavioral progress. The hypo-metabolism of glucose is supported in monkey model of AD induced by STZ. The above described facts are linked with insulin and IR function in the brain of SAD patient. STZ exerts pleiotropic effects on brain in the process of inducing SAD. IR are dispersed variably in different regions of brain including hippocampus, cerebral cortex, hypothalamus and olfactory bulb. Impairment of insulin signaling is the true cause for SAD, regardless of diabetic or non-diabetic status.

In physiological condition insulin binds to IR and induces auto-phosphorylation, consequently activates several secondary messengers which lead to different physiological cascades. Insulin acts on IR and increases IR substrate, activation of phosphotydilinositol-3 kinase (PI3K) and activation of protein kinase B (PKB). PKB involved in glucose metabolism and inactivation of GSK-3β. Glucose metabolism is controlled by insulin through metabolizing glucose into fructose-6-phosphate then acetyl CoA. Acetyl CoA is essential for learning and memory process.

STZ peripheral administration enters beta cells through GLUT2 transporter, the same transporter which is used by STZ when administered centrally. This is supported by the presence of GLUT2 transporter in distinct regions of rat brain. STZ passes through GLUT2 and causes DNA alkylation, leading to beta cell destruction. STZ ICV administration causes altered GSK-3β and reduced expression of brain derived neurotrophic factor, finally synaptic dysfunction and insulin resistance. The final
cascade that occurs due to STZ is IR degradation. IR degradation leads to signal impairment of IR substrate which leads to deactivation of PI3K. Down regulation of PI3K with PKB decreases glucose metabolism and activates GSK-3β. Decreased glucose metabolism or uptake leads to increase in the production of toxic advanced glycation end products which further leads to tau hyperphosphorylation. Activated GSK-3β also causes tau hyperphosphorylation and formation of NFT.

Deposition of amyloid plaques is another key feature seems to be consistent with late onset of AD i.e., SAD in STZ model. Three months followed the STZ ICV administration forms amyloid plaques and progress with age in non-transgenic SAD animal. IR degradation causes increased insulin levels in the brain, contribute to accumulate Aβ. This may be due to accelerated APP processing and down regulation of insulin degrading enzyme (IDE). Recent reports suggest that increased insulin level inhibit IDE further leading to Aβ accumulation. Controversially decrease in insulin levels followed by insulin resistance in central nervous system (CNS) impairs clearance of Aβ. Taken together both high and low level of brain insulin may contribute to SAD, it should be maintained optimal for its protective role against SAD.

Unlike the classic peripheral actions, insulin exhibits diverse action in the CNS. The presence of insulin and IRs in hippocampus and cerebral cortex testifies for its role in cognitive functions like learning and memory. There are several reports showing that STZ central administration deteriorates memory function. This impaired memory function in STZ model is largely attributed to dysfunctional insulin signaling resulting in depletion of cAMP binding protein, Akt, and IDE, and increase in Aβ levels. Blanchard and Duncan have shown that insulin administration ameliorates scopolamine-induced amnesia in radial arm maze (RAM) task. Craft et al. tried clinically by administering insulin to AD patients and to show memory improvement at different doses of insulin.
Insulin activates IRs and leads to gene expression through Scr homology and collagen containing mitogen-activated protein (MAP) kinase pathway that is required for long term memory. IR activation also facilitates short-term memory storage through phospholipase C (PLC) and protein kinase C (PKC) pathway. Intracerebroventricular STZ exacerbates cognitive deficits, tau phosphorylation and neuroinflammation in 3×Tg-AD mice. SAD induced by STZ in monkey model demonstrates the impairment of insulin-like growth factor (IGF) genes in different regions of the brain including hippocampus, frontal cortex and cerebrum. Mitochondrial abnormalities including membrane potential and adenosine triphosphate (ATP) content are also identified in SAD induced by STZ.

2.2. Review on relation between T2D and AD

There are numerous features that are common in T2D and AD. This is well supported by many of the researchers. Following are some of the important literature that reveal the relation of T2D and AD. The risk of developing AD increases with T2D. Fu et al. recently showed amylin receptor as a common pathophysiological target in AD and T2D. Amyloidgenic peptide Aβ plays a key role in AD forming insoluble aggregates in the brain, and in T2D, amylin forms aggregates in the pancreas. There are some common properties in Aβ and amylin which is beneficial for the treatment of AD and T2D. Drugs used to ameliorate hyperglycemia and insulin resistance may also have beneficial effects in AD. Investigations on metformin, thiazolidinediones, leptin, GLP-1 therapies and insulin have shown promising results. Supporting the above statement Yang and Song, Han and Li, and Maher and Schubert discuss the molecular link between AD and T2D. There has been much concern regarding the role of dietary sugars in the development of T2D; recent findings suggest that high-sugar diets can also lead to cognitive impairment predisposing to AD. Some of the authors discuss the
commonalities between AD and T2D.\textsuperscript{30, 95-99} Bartl et al.\textsuperscript{100} explore the common link between T2D and AD. Consistently Malek-Ahmadi et al.\textsuperscript{101} explore the increase of AD pathology with T2D. In animal model of T2D and AD, similar behavioral, cognitive and vascular anomalies are observed.\textsuperscript{87} Recent evidence suggest that patients with T2D have a 50-70% increased risk of developing AD, which reveals the increase in tau phosphorylation in T2D mice.\textsuperscript{102} Ke et al.\textsuperscript{103} showed the elevation of tau pathology in experimental diabetes mellitus. Supporting the above report Bitel et al.\textsuperscript{104} also showed the development of Aβ and tau pathology in a diabetic rabbit. This is further supported by Ma et al.\textsuperscript{105} by showing the hyperphosphorylation of tau protein in T2D + AD animals. At the same time additionally, Accardi et al.\textsuperscript{106}, Kroner,\textsuperscript{107}, de la Monte and Wands,\textsuperscript{108} and Lester-Coll,\textsuperscript{109} call AD as type-3-diabetes. Abnormal protein processing, abnormalities in insulin signaling, deregulated glucose metabolism, oxidative stress, the formation of advanced glycation end products, and the activation of inflammatory pathways are features common in T2D and AD.\textsuperscript{110,111} GSK-3 inhibitors may be a key target to develop therapeutic agents for AD as well as T2D.\textsuperscript{112} The pathology and treatment shared by T2D and AD gives the way for developing novel therapeutic agents for both the disorders.\textsuperscript{113} Arab et al.\textsuperscript{114} hypothesized about the diabetes treatment in preventing AD. The major interrelations between T2D and AD are insulin signaling, glucose metabolism and mitochondria.\textsuperscript{115} Adding to that, Moreira,\textsuperscript{116} integrated the role of mitochondria, oxidative stress and insulin in AD and diabetes. The role of mitochondria as therapeutic target in AD and T2D is detailed in Moreira et al.\textsuperscript{117} Takeda et al.\textsuperscript{118} also discussed about Aβ peptide, insulin signaling and neuronal function as molecular mechanisms linking between T2D and AD. Supporting that Zhao and Townsend,\textsuperscript{119} reviewed the insulin resistance and amyloidogenesis as common molecular foundation for T2D and AD. Holscher,\textsuperscript{120} propose insulin signaling impairment in the
brain as an alternative model for AD. Consistently, Sato et al.\textsuperscript{121} reviewed the role of insulin signaling in the interaction of AD and T2D. Adding to it, Liu et al.\textsuperscript{122} have shown deficient brain insulin signaling pathway in AD and diabetes. There are animal models which provide link between T2D and AD.\textsuperscript{123}

\textit{2.3. Review on role of GLP-1 and DPP-4 in AD}

GLP-1 is the major incretin hormone secreted from the L-cell of the gastrointestinal cell in response to ingested food. It is responsible for various physiological functions including beta-cell neogenesis, insulin biosynthesis and glucose-dependent insulin secretion. Regulation of glucose metabolism and maintaining blood glucose by GLP-1 in T2D patients been exploited. However, a small amount of GLP-1 is also produced in the brain, especially from the nucleus of solitary tract and caudal brain stem.\textsuperscript{124} GLP-1 possesses neuroprotective actions in neurodegenerative disorders including Alzheimer’s and Parkinson’s disease. GLP-1 together with its receptors expressed in hippocampus where the neurons are more vulnerable for neuronal degeneration.\textsuperscript{125}

GLP-1 is rapidly degraded by DPP-4, which makes GLP-1 inactive and leads to very short half-life of the intact hormone\textsuperscript{126}. Bell et al.\textsuperscript{127} first identified GLP-1, and its analogs, exendin and liraglutide are developed which are resistant to DPP-4 inactivation. These GLP-1 analogs are used for the treatment of T2D are well reported for the amelioration of AD. The present trend is turning towards activation of GLP-1 receptors in AD. GLP-1/GLP-1 agonist binds to G-protein coupled receptor and activates adenylyl cyclase, PKC and MAP kinase, there by protects neurodegeneration.\textsuperscript{125} Similar to GLP-1 functions in T2D, it is also responsible for neuronal cell protection, proliferation and differentiation.\textsuperscript{124}
Perry et al.\textsuperscript{128} observed the effects of GLP-1 in protecting neurons and reducing Aβ peptides. Consistently Biswas et al.\textsuperscript{129} reported the prevention of neurodegeneration caused by nerve growth factor deprivation in cultured PC12 neuronal cells by GLP-1. Following that, the neuroprotection by GLP-1 was confirmed in GLP-1 knockout mice, which shows cognitive deficits in behavioral tests and synaptic plasticity impairment.\textsuperscript{130} Li et al.\textsuperscript{131} showed that GLP-1 receptor activation by exendin-4 reduces Aβ toxicity and oxidative stress in cultured neurons and further in STZ 3×Tg-AD mice, exendin-4 reduces APP and Aβ. Consistently another GLP-1 agonist (Val8)-GLP-1 prevents spatial learning deficits and reduces hippocampal impairment induced by Aβ1-40.\textsuperscript{132} Similarly (Val8)-GLP-1 reduces tau phosphorylation and improves cognitive behavior in STZ injected rats.\textsuperscript{37} The result of (Val8)-GLP-1 is further supported by Gengler et al.\textsuperscript{38} where (Val8)-GLP-1 prevents neurodegeneration by ameliorating synaptic plasticity. In this pathway, Bak et al.\textsuperscript{133} predicts that GLP-1 have both neurotrophic and neuroprotective effects, and may be a novel therapeutic approach for treating AD. McClean et al.\textsuperscript{134} proved the administration of liraglutide, a GLP-1 agonist, reduced oligomerization of plaques, neuro-inflammation and brought back the cognitive ability in APP/PS1 mice. Recently Long-Smith and colleagues\textsuperscript{135} demonstrated that AD patients treated with liraglutide, a GLP-1 agonist, mitigated AD pathology including reduction of plaque load and astrocytosis.

GLP-1 is rapidly degraded by a serine peptidase called DPP-4, which is expressed in body fluids and various tissues. Different approaches have been implemented to extend the GLP-1 activity for the treatment of T2D.\textsuperscript{136} DPP-4 inhibition is one such approach protects insulinotropic action of GLP-1. Research on enhancing GLP-1 using DPP-4 inhibitors for the treatment of AD is rising and is supported by the following studies. Metformin, a drug well known for the treatment of T2D is proved to inhibit DPP-4.\textsuperscript{137}
Adding to that, it also shows reduced tau phosphorylation in vitro and in vivo.\textsuperscript{138} Sitagliptin, the first DPP-4 inhibitor available in the market for the treatment of T2D is also proved to mitigate AD in a long term therapy.\textsuperscript{43} Consistently in our previous attempts, it has been confirmed saxagliptin\textsuperscript{44} and vildagliptin\textsuperscript{45} ameliorate AD in a STZ model.

2.4. Review on phytochemistry and pharmacology of plants used in the present study

Ayyanar and Subash-Babu,\textsuperscript{139} described the existing information on botany, phytochemical constituents, traditional uses and pharmacological actions of \textit{E. jambolana} Skeels (jambolan). The plant has been viewed as an antidiabetic plant since it became commercially available several decades ago. The plant is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin. The seeds are claimed to contain alkaloid, jambosine, and glycoside jambolin or antimellin, which halts the diastatic conversion of starch into sugar.

Baliga et al.\textsuperscript{140} reviewed the aspects of \textit{E. jambolana} and also addresses the lacunas in the existing knowledge. \textit{E. jambolana} Lam. commonly known as black plum or Jamun, a plant native to India. Annually the trees produce oblong or ellipsoid fruits (berries). They are green when raw and purplish black when fully ripe. The ripe fruits are sweetish sour to taste and are used to prepare health drinks, squashes, juices, jellies and wine. Berries contain carbohydrates, minerals and the pharmacologically active phytochemicals like flavonoids, terpenes, and anthocyanins. Jamun is a plant with known ethnomedicinal uses. Before the discovery of insulin, Jamun was useful in the treatment of diabetes and is an integral part in the various alternative systems of medicine. Scientific studies have shown that the various extracts of Jamun possess a range of pharmacological properties such as antibacterial, antifungal, antiviral, anti-genotoxic, anti-inflammatory, anti-ulcerogenic, cardioprotective, anti-allergic, anticancer, chemopreventive,
radioprotective, free radical scavenging, antioxidant, hepatoprotective, anti-diarrheal, hypoglycemic and antidiabetic effects.

Maurya et al.\textsuperscript{141} isolated five new flavonoid C-glucosides, 6 - hydroxyl - 2 - (4-hydroxybenzyl) - benzofuran - 7 - C - beta - d - glucopyranoside, 3 - (alpha - methoxy - 4 - hydroxybenzylidene) - 6 - hydroxybenzo - 2(3H) - furanone - 7 - C - beta - d - glucopyranoside, 2 - hydroxyl - 2 - p - hydroxybenzyl - 3(2H) - 6 - hydroxybenzofuranone - 7 - C - beta - d - glucopyranoside, 2 - hydroxyl - 2 - p - hydroxybenzyl - 3(2H) - 6 - hydroxybenzofuranone - 7 - C - beta - d - glucopyranoside, 8 - (C - beta - d - glucopyranosyl) - 7, 3', 4' - trihydroxyflavone, 1, 2 – bis (2, 4 - dihydroxy, 3 - C - glucopyranosyl) - ethanedione, C -beta - d - glucopyranosyl - 2, 6 - dihydroxyl benzene and sesquiterpene, from an aqueous extract of the heartwood of \textit{P. marsupium}.

Devgun et al.\textsuperscript{142} explored \textit{P. marsupium} description, traditional uses, extraction methods, chemical constituents, pharmacological activity and commercial importance. The role of \textit{P. marsupium} as anti-diabetic has been very well established. Its extract has been prepared using many methods like infusion, maceration, decoction and percolation. Several chemical constituents like pterostilbene, (-)-epicatechin, pterosupin, marsupsin, etc., have been identified and isolated. \textit{P. marsupium} extract also shows promising results in cataract and hypertriglyceridaemia. This plant also finds its use as cardiotonic and hepatoprotective agent. Studies have also been reported to demonstrate its ability as a specific COX-2 inhibitor.

Badkhane et al.\textsuperscript{143} revealed that the biological activities and medicinal properties of isolated compounds of \textit{P. marsupium}, pharmacological actions of the \textit{P. marsupium} extracts, clinical studies and plausible medicinal applications along with their safety evaluation. \textit{P. marsupium} is a popular Indian medicinal plant and since many years this has been used commonly in ayurvedic system of medicine. The plant has been found to possess diverse number of biological activities. The \textit{P. marsupium} tree has some
medicinal property and is thus commercially exploitable. During the last five decades, apart from the chemistry of the *P. marsupium* compounds, considerable progress has been achieved regarding the biological activity and medicinal applications of *P. marsupium*. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products.

Kanetkar et al.\textsuperscript{144} reviewed the molecular perspective linking the common medicinal plants to the most common metabolic disorders. *G. sylvestre* is regarded as one of the plants with potent anti-diabetic properties. This plant is also used for controlling obesity in the form of gymnema tea. The active compound of the plant is a group of acids termed as gymnemic acids. It has been observed that there could be a possible link between obesity, gymnemic acids and diabetes.

Porchezhian and Dobriyal,\textsuperscript{145} explained the chemistry and pharmacology of *G. sylvestre*. Extracts of this plant are widely used in Australian, Japanese, Vietnamese and Indian folk medicine. Gymnema preparations have a profound action on the modulation of taste, particularly suppressing sweet taste sensations. It is used in the treatment of diabetes mellitus and in food additives against obesity and caries. Anti-allergic, antiviral, lipid lowering and other effects are also reported. From a technological point of view, considerable efforts have been made to mask the bitter taste of gymnema preparations.

Kamble et al.\textsuperscript{146} developed and evaluated *G. sylvestre* extract-loaded nonionic surfactant-based niosomes. In different approach Kamble et al.\textsuperscript{148} performed pharmacokinetic evaluation of gymnemagenin in rat plasma using high performance liquid chromatography (HPLC). At the same time gymnemagenin was also estimated in gymnema sylvestre and marketed formulations using HPLC.\textsuperscript{149}