Effect of Vildagliptin on Cognitive Deficits in an Experimental Model of Alzheimer's Disease

Devasrita Dash[1]*, Laxminarayana Bairy Kurady[2] and Bharti Chogtu[3]

[1]Department of Pharmacology, Melaka Manipal Medical College (Manipal Campus),
Manipal Academy of Higher Education (MAHE), Manipal-576104, Karnataka, India.
[2]Department of Pharmacology, Ras Al Khaimah College of Medical Sciences,
Ras Al Khaimah-11172, UAE.
[3]Department of Pharmacology, Kasturba Medical College, Manipal Academy
of Higher Education (MAHE), Manipal-576104, Karnataka, India.
*Corresponding Author E-mail: devasrita@gmail.com

Type 2 diabetes is considered a pivotal risk factor for Alzheimer's disease (AD). Aluminium chloride induces hippocampal structural & functional abnormality and causes neurodegeneration. Our study evaluated the effects of vildagliptin on spatial memory, cholinergic activity, and neuronal survival in cornu ammonis 3 (CA3) region of hippocampus in an aluminium chloride-induced AD in male Wistar rats. Male Wistar rats were randomly divided into five groups. All animals except normal control were exposed to aluminium chloride (17 mg/kg/day) and group 3, 4 and 5 were simultaneously received rivastigmine (6 mg/kg/day), vildagliptin (5 mg/kg/day and 10 mg/kg/day) treatment respectively for 30 days. Assessment of spatial memory was followed by estimation of acetylcholinesterase (AChE) activity and quantification of neuronal cell count in CA3 region of hippocampus. Vildagliptin improved spatial memory, decreased acetylcholinesterase levels, and improved neuronal count in CA3 region of hippocampus through multimodal approach. Vildagliptin treatment significantly attenuated aluminium chloride-induced cognitive deficits. It may serve as a promising candidate in the management of concomitant AD and type 2 diabetes mellitus (T2DM).

Keywords: Alzheimer's disease; Aluminium chloride; CA3 region;
Cognition; Type 2 diabetes; Vildagliptin.

Alzheimer's disease (AD) is a slowly progressing brain disorder with deleterious effects on memory, cognition, and behavior. Neuropathological hallmarks of AD include senile plaques[1,2], neurofibrillary tangles[1,2], brain atrophy, cholinergic dysfunction[1,2], and neurodegeneration[1,2]. AD is the most common form of dementia[3] in the older population and poses a significant economical and social burden to society. The predominance of AD doubles every 5 years[4], after the age of 65[4]. By the year 2050, the number of people aged 65 and above with Alzheimer's dementia, is projected to reach 12.7 million[5].

Current drug therapy for AD improves cognitive impairment, only through symptomatic relief. Hence, there has been a constant need for drugs, that will delay, prevent and/or reverse cognitive and behavioral changes seen in AD.

Evidence suggests that AD is multifactorial in origin. Over the years, T2DM has been considered an important risk factor for AD. Insulin in the brain is essential for the regulation of

memory and cognitive functions. Commonalities shared by T2DM and AD are as follows: insulin resistance, neuronal and synaptic damage, amyloid β (Aβ) deposition, tau hyperphosphorylation, brain atrophy, oxidative stress, apoptosis, inflammation, ApoE4, cardiovascular disease, and higher cholesterol levels[6–9]. Chronic hyperglycemia also induces similar neurodegenerative changes. The bidirectional link between the two diseases and the lack of disease-modifying drugs for AD makes it imperative to find new drugs that will target comorbidities and improve cognitive impairment at a cellular level.

Dipeptidyl peptidase-4 (DPP-4) inhibitors have dramatically changed the treatment aspect of diabetes mellitus [10]. Vildagliptin, a dipeptidyl peptidase-4 inhibitor affords benefit in diabetic patients by increasing insulin secretion through GLP-1 mediated mechanism. GLP-1 in the brain acts via GLP-1 R (receptors) located in the cortex[11], hippocampus[11], and cerebellum [11]. As reported, GLP-1 can cross the blood-brain barrier[12], and prevent neuronal damage through central anti-inflammatory and antiapoptotic effects[13]. It decreases amyloid protein precursors (APP), Aβ levels, hyperphosphorylated tau protein levels, brain inflammation and ameliorates brain mitochondrial dysfunction[11,14].

Aluminium chloride on administration, crosses BBB, accumulates in the hippocampus, frontal cortex and induces neurodegenerative changes (AD). It can induce oxidative stress, neuroinflammation, insulin resistance, synaptic & neuronal damage, cholinergic dysfunction, protein self-aggregation, misfolding, forming senile plaques, neurofibrillary tangles, which are classical hallmarks of Alzheimer's disease (AD)[15–28]. With this background, our study was planned to explore the effects of vildagliptin in attenuating neurodegenerative changes in AD alone and with concomitant T2DM on an experimental model of aluminium chloride-induced AD.

MATERIALS AND METHODS

Animals

Three months aged, male Wistar rats, weighing 140–200 g, were procured from Central Animal Research Facility (CARF) of Manipal University, Manipal. Animals were maintained under $23 \pm 2°C$ and $50 \pm 65\%$ humidity. A 12 h light/dark cycle was maintained. Rats were maintained on normal food and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee, Kasturba Medical College, Manipal, and was completed as per the guidelines stated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Drugs and chemicals

Drugs used were obtained as follows: Aluminium chloride ($AlCl_3$) from Merck life sciences private limited, metformin from Franco-Indian Pharmaceuticals Pvt Ltd., rivastigmine from Sun Pharmaceutical Industries Ltd., and vildagliptin from Novartis Healthcare Pvt Ltd. Analytical grade chemicals and reagents were used for the study.

Experimental design

Thirty animals were divided into 5 groups with 6 animals each. They were subjected to drug administration as mentioned in Table 1.

Aluminium chloride model of Alzheimer's disease

Induction of disease was done through oral administration of aluminium chloride (17 mg/kg), once daily for 30 days [29,30].

Spatial memory assessment through Morris water maze (MWM)

Spatial learning and memory were assessed using water maze. The apparatus consisted of a round tank of 1.8 m in diameter placed in a room illuminated by poorly lit white fluorescent lamps with distant visual stimulus. The tank was half-filled with water and made cloudy by adding milk. Four points were assigned N, E, S, W along the circumference of the pool dividing the tank into 4 equal quadrants. A plexiglass escape platform was immersed 2 cm underneath the water surface and paced at mid-point of any one quadrant. The positioning of the escape platform was maintained through the acquisition trials. Experiment was conducted for 5 days, with 4 days of acquisition trials followed by retention test (probe trial) on 5th day of the test. All animals underwent 1 session of 4 trials/day for 4 consecutive days[31]. Starting points for all trials were randomized. Animals incapable of discovering the platform within 60 s were directed to locate it by us. Animals remained on it for 30 s. The interval between trials was 60

s. On the 5th day, the platform was taken out of the tank, and each animal was allowed to swim for a 60 s[31,32]. Data were expressed as escape latencies and time spent in the target quadrant.

Sampling and Preparation of Brain Tissue

After MWM was conducted, animals were sacrificed by cervical decapitation. The brain was dissected and washed with ice-cold phosphate buffer (PBS- pH 7.4) thoroughly. Hippocampus was identified and homogenized in PBS (10% w/v, pH 7.4). Centrifugation of the homogenate was done at 4°C for 10 min. at 3000 rpm. Supernatant was carefully separated and was utilized for estimating the levels of acetylcholinesterase.

Acetylcholinesterase assay

Acetylcholinesterase activity was determined as per Ellman et al. [33] method with necessary modifications. Enzyme activity was read spectrophotometrically at 420 nm and calculated based on the changes in absorbance/min[32].

Histopathological study

Brain extraction was done after perfusion with ice-cold phosphate-buffered saline and stored in 10% formalin. Brain tissue was dehydrated in varying grades of alcohol and xylene. Tissue was embedded with paraffin wax and coronal sections of 5 μm thickness were sliced from dorsal hippocampus with a rotatory micrometer (Leica RM2245, Leica microsystems, Germany). Each section was mounted on air-dried gelatinized slides[34]. Every 20th section was selected and 25-30 sections from each slide were stained and mounted on air-dried gelatinized slides. Cresyl violet stain was prepared using a standard protocol[34]. Each slide was stained with cresyl violet (0.1%) and observed under a light microscope (10X and 40X magnification).

Neuronal quantification in CA3 region of hippocampus

Quantification of healthy neurons in CA3 region of hippocampus was done in a light microscope under 10X magnification (Olympus BX43 microscope with attached DP21 digital camera, Germany). Counting was done by using ImageJ software v1.53.o (National Institute of Health, available free online). Six sections from each rat were used for the counting. The cell counts were expressed as the number of cells per unit

Table 1. Study design.

Groups	Drugs, dose, and route of administration (for 30 days)
1. Control	Distilled water orally
2. Disease model	Aluminium chloride (17 mg/kg) orally
3. Disease model treated with rivastigmine	Aluminium chloride (17 mg/kg) + rivastigmine (6 mg/ kg) orally
4. Disease model treated with vildagliptin (Dose 1)	Aluminium chloride (17 mg/kg) + vildagliptin (5 mg/ kg) orally
5. Disease model treated with vildagliptin (Dose 2)	Aluminium chloride (17 mg/kg) + vildagliptin (10 mg/ kg) orally

Table II. Reversal of AlCl$_3$-induced elevation of acetylcholinesterase activity by vildagliptin

Groups	Acetylcholinesterase activity(ìmol/mg/min)
Control	$0.03078 \pm 0.0021^*$
Disease model (DM)	$0.08091 \pm 0.0140^\#$
DM + Rivastigmine	$0.03568 \pm 0.0015^*$
DM + Vildagliptin dose 1	$0.05120 \pm 0.0030^*$
DM + Vildagliptin dose 2	$0.03043 \pm 0.0013^*$

One way ANOVA followed by Tukey's post-hoc test showed *vs DM (*$p<0.05$), #vs Control (#$p<0.05$).; n=6

length of the cell (cells/200 μm) as described by [35]. Well-rounded cells with distinct nuclei and no pyknosis were counted as surviving cells.

Statistical analysis

For analyzing the data, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Data analysis was done in SPSS software (version 22). Results were expressed as mean ± SEM. P-value less than 0.05 was set as the level of significance.

RESULTS

Effect of vildagliptin on AlCl₃-induced spatial memory deficits

Effect of aluminium chloride and drug treatments on spatial learning memory was assessed by recording escape latencies and time spent in target quadrant through water maze test (figure I and II).

During acquisition trial, control groups and drug treated groups showed improvement in escape latencies. On day 4 of acquisition, control groups and drugs treated groups showed a significant decrease in escape latency to locate the platform in the target quadrant in comparison with aluminium chloride group ($p<0.05$).

Probe trial demonstrated aluminium chloride group exhibited an escape latency of 45.3 seconds in comparison with control group 2.35 seconds indicative of reduced retention of spatial memory($p<0.05$). Rivastigmine treated group showed improved escape latency at 1.49 seconds (in comparison with aluminium chloride group on day 5; however, the spatial memory was better restored in vildagliptin dose 2 treated groups with an escape latency of 1.47 seconds ($p<0.05$).

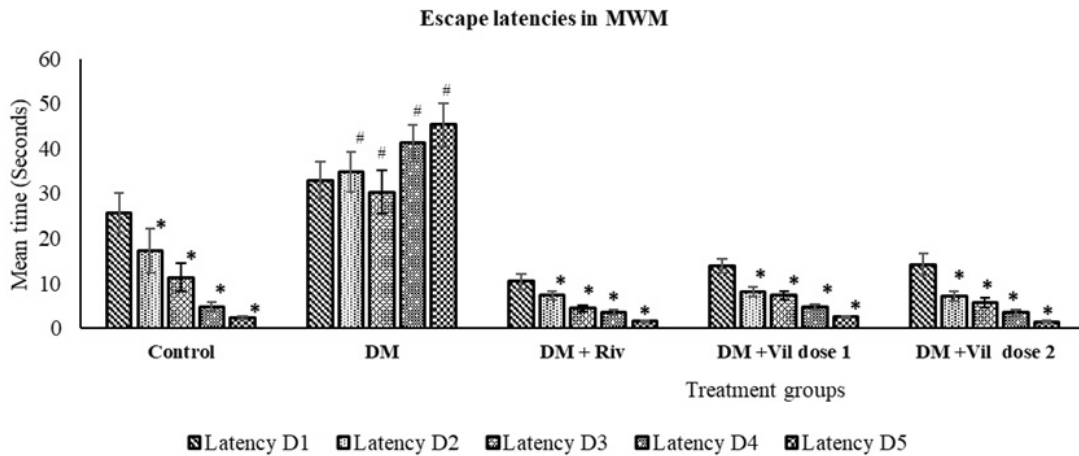Throughout the acquisition trials, a significant increase in time spent in the target

Fig. I. Effect of drugs on escape latencies of rats in MWM
One way ANOVA followed by Tukey's post-hoc test showed *vs DM (*$p<0.05$), #vs Control (#$p<0.05$), n= 6
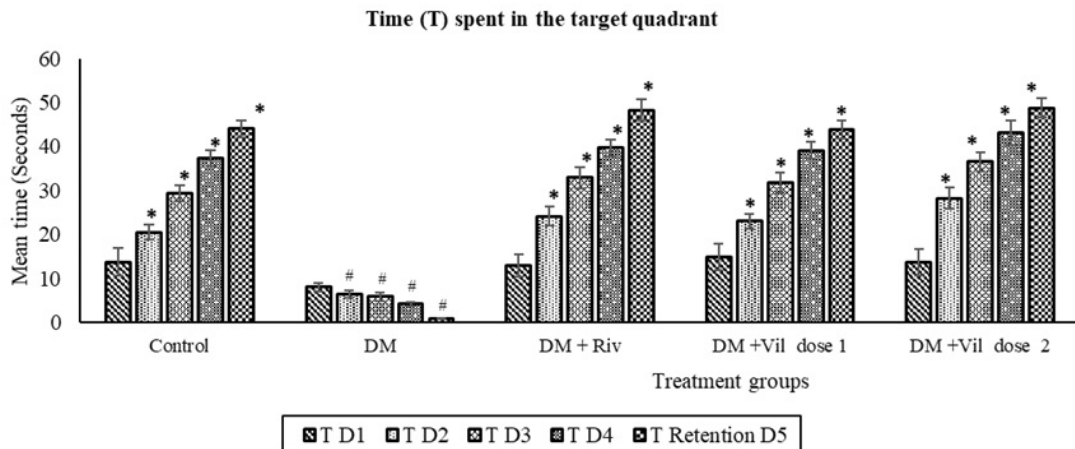

Fig. II. Effect of drugs on time spent in the target quadrant in MWM
One way ANOVA followed by Tukey's post-hoc test showed *vs DM (*$p<0.05$), #vs Control (#$p<0.05$), n=6

quadrant was observed in control and drug treatment groups on day 4 in comparison with aluminium chloride group (p<0.05). A significant decline in time spent in the target quadrant was observed in aluminium chloride group throughout the acquisition trials (p<0.05).

Time spent by aluminium chloride group in target quadrant on the retention trial day was 0.82 seconds indicative of severe decline in spatial learning and memory retention (p<0.05) Rivastigmine with 48.2 seconds, vildagliptin treated groups dose 1 with 43.8 seconds, and dose 2 with 48.7 seconds showed a significant increase in time spent in the target quadrant compared to aluminium chloride group indicating better spatial learning and memory retention (p<0.05).
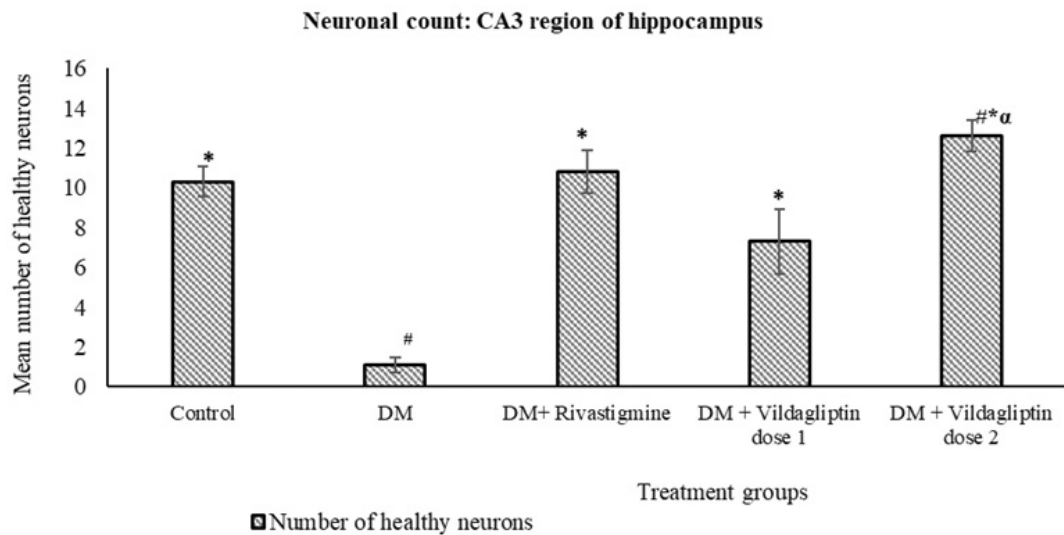

Fig. III. Change in the number of CA3 hippocampal neurons in male Wistar rats post aluminium chloride, rivastigmine, and vildagliptin administration

One way ANOVA followed by Tukey's post-hoc test showed *vs DM (*p<0.05), #vs Control (#p<0.05), Vil2 vs Vil1 (α p value < 0.05) respectively; n=6
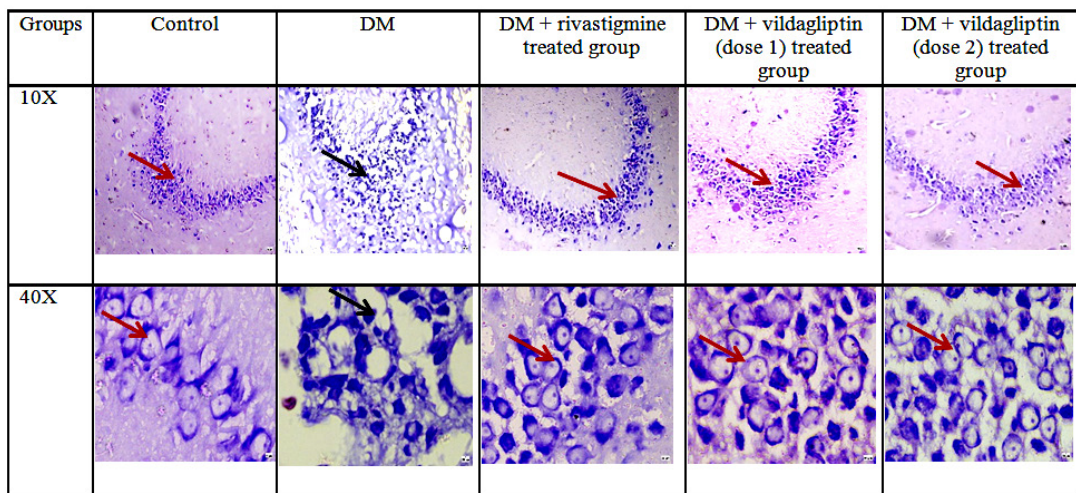

Fig. IV. Histopathological changes of CA3 region of hippocampus. Cresyl-violet-stained slides were observed under light microscope in 10x/40x magnification and representative pictures were depicted above A-10x, B-40x. Red arrow-healthy neurons, Blank arrow-degenerated neurons

Reversal of AlCl₃-induced elevation of acetylcholinesterase activity by vildagliptin

Four weeks of aluminium chloride administration significantly increased AChE activity in comparison with control ($p<0.001$). Rivastigmine and vildagliptin reduced the AChE activity in comparison with aluminium chloride group ($p<0.05$) as shown in Table 2.

Effect of vildagliptin on histopathological changes and neuronal cell count in CA3 region of the hippocampus

Photomicrographs of CA3 region observed in different groups under light microscope at 10x and 40x magnification are depicted in figure IV. Pyramidal cells in control group of animals presented with well-rounded cells with clear cytoplasm and distinct nucleus. However, aluminium chloride group of animals presented with irregularly shaped hyper-dense stained cells, pyknotic nucleus, lack of well-defined boundary between cytoplasm and nucleus, cellular dispersions with formation of pockets in between cells representing necrotic cells (dead cells). Pyramidal cell count showed a significant decrease compared to control group ($p<0.05$). Rivastigmine and vildagliptin treated groups, showed a significant improvement in cellular morphology with visibility of clear cytoplasm and distinct nucleus. Drug treatments increased healthy cell count compared to aluminium chloride group ($p<0.05$). Vildagliptin dose 2 showed a significant increase in healthy cell count compared to vildagliptin dose 1 ($p<0.05$) as shown in figure III.

DISCUSSION

Alzheimer's disease is multifactorial in origin and current drugs focus on restoring the cholinergic dysfunction. T2DM is a risk factor for AD and both chronic conditions share a bidirectional link between their pathophysiology. Few studies have labelled AD as type 3 diabetes/ brain diabetes[36,37]. We hypothesized that antihyperglycemic drug like DPP-4 inhibitors can have a role in amelioration of the neurodegenerative changes seen in AD.

The cholinergic neurons from the nucleus basalis of Meynert undergo degeneration in Alzheimer's disease and contribute to the memory loss [38,39]. Hence, AChE activity is a key determinant of AD. Our study showed an increase in acetylcholinesterase activity in aluminium chloride group in comparison with control ($p<0.001$). Vildagliptin and rivastigmine groups showed a significant decline in acetylcholinesterase activity in comparison with aluminium chloride group suggesting that there is an improvement in cognition. Rivastigmine interacts with the esteratic site of brain AChE and increases the availability of brain acetylcholine thereby restoring cholinergic dysfunction [40]. It also inhibits AChE in plaques and tangles with the same potency as those in neurons and axons and thus compensates the cholinergic deficit caused by aluminium [41–43]. In a transgenic mice model of AD, rivastigmine has modified the levels of several shedding proteins and directed APP processing towards the α-secretase pathway [43]. Overall, through these actions, rivastigmine enhances cholinergic functions in the brain and restores functions of neural cells thereby mitigating cognitive deficits. GLP-1 reduces AChE activity, possibly through activation of its central receptor[44]. Combination of vildagliptin and galantamine has shown a marked decrease in AChE activity[45]. Although, there is limited evidence to point a finger at the exact mechanism behind decrease in AChE activity with vildagliptin in our study.

Hippocampal degeneration, impaired neuronal network, reduced neurogenesis[46], and cognitive[46] deficits are seen in AD. CA3 is the largest area in the hippocampus which regulates major cognitive functions and is more prone to oxidative stress and injury. Further, oxidative stress can initiate neuroendocrine alterations within the amygdala, including amygdalar hyperactivity and dendritic shrinking [47]. Histopathological examination of cresyl violet-stained hippocampal slices of aluminium chloride group in present study have shown presence of pyknotic cells, densely stained pyramidal cells with irregular morphology with cellular dispersions and loss of cell bodies with their Nissl's substance connoting neuronal degeneration. Earlier studies have reported similar morphological irregularities in pyramidal cells with aluminium chloride exposure[35,48–50]. A significant decline in pyramidal cell count in CA3 region of the hippocampus has been observed in aluminium chloride group in this study. The extent of neuronal degeneration seen in CA3 region is high compared to other areas of hippocampus. Aluminium chloride

induced hippocampal damage has been reported in other studies as well [49].

Vildagliptin and rivastigmine treatment dramatically mitigated cellular death and damage and improved pyramidal cell count in CA3 region. Treatment with vildagliptin dose 1 and dose 2 effectively caused a significant increase in neuronal cell count and ameliorated neurodegenerative changes seen in AD. Dose 2 proved to be more effective against AD compared to dose 1. Pyramidal neurons in CA3 region of the hippocampus express GLP-1R [51]. GLP-1 influences brain metabolism[52] and stimulates neuritic growth in CNS neurons[52]. GLP-1 exerts a protective effect against excitotoxic cell death and toxic $A\beta_{1-42}$ [53]. i.v. administration of GLP-1 has reduced nerve cell damage and improved learning and memory in a mice model [54]. Several studies support our findings that vildagliptin improves pyramidal cell count and reverses cognitive deficits induced by aluminium chloride through its neuroprotective effect and probably this also compliments its antiapoptotic effect. Decreased levels of AChE as reported in our study has a potential to improve cholinergic function and ameliorate neuronal death in CA3 of the hippocampus. A study on total and phosphorylated tau in a combined model of AD and T2DM also supports our findings[55].

Aluminium chloride group has shown a significant increase in escape latency and a significant decrease in the time spent in the target quadrant. Research findings in MWM are suggestive of impairment of hippocampal functioning and cognition due to aluminium chloride exposure. Vildagliptin and rivastigmine treatment have improved hippocampal functions as evident through decreased escape latency and significantly more time spent by these animals in the target quadrant.

Chronic aluminium chloride exposure interferes with insulin signaling and induces brain insulin resistance. Studies mention that insulin can alter AChE activity and influence cholinergic functions [56]. Brain insulin regulates cellular apoptosis, neuronal proliferation, glial cell activity, amyloid-beta protein clearance, tau protein phosphorylation, synaptic plasticity, and memory formation. Impairment in signaling will interfere with these metabolic processes and induce the formation of senile plaque, neurofibrillary tangles, atrophic changes, neuronal damage, and death in the brain leading to irreversible memory and cognitive damage. These neuropathological features represent classical hallmarks of AD and are shared by T2DM [57]. Mitochondrial dysfunction also contributes to insulin resistance and is shared by both diseases [58]. Mitochondrial dysfunction induces energy deficiency[59] and interferes with metabolic processes in the body[60].

Studies have reported that GLP-1 has direct central actions on the brain, and possesses neurotrophic effects [54,61–65]. DPP-4 inhibition improves glucose metabolism[66] through the upregulation of insulin secretion[66] and the suppression of glucagon release [66]. Vildagliptin enhances glucose-dependent insulin secretion[67] and lowers blood glucose[67] in T2DM through GLP-1[67]. Vildagliptin has prevented high-fat diet induced brain and hippocampal dysfunction in insulin-resistant rats[61]. Our research findings and available evidence points toward a multimodal approach of vildagliptin in attenuating neurodegenerative changes seen in Alzheimer's disease. Treating AD with an antidiabetic drug may decrease the economic burden, and slow disease progression. It may improve the quality of life of patients as well as caregivers. However, studies are required to deduce the exact role vildagliptin plays in Alzheimer's disease.

CONCLUSION

This study demonstrated that vildagliptin: i) improved spatial learning and memory retention ii) increased availability of acetylcholine and iii) increased pyramidal cell count in CA3 regions of the hippocampus, iv) attenuated neurodegeneration induced by aluminium chloride in Wistar rats. Based on our findings we conclude that vildagliptin may serve as a potential drug for attenuating neuropathological changes in AD.

ACKNOWLEDGMENT

Conflict of Interests
The authors declare that they have no conflict of interests.

Fundings

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

REFERENCES

1. Perl, D. P. Neuropathology of Alzheimer's disease. *Mt. Sinai J. Med.* **77**, 32–42 (2010).
2. Carlesimo, G. A. & Oscar-Berman, M. *Memory Deficits in Alzheimer's Patients: A Comprehensive Review. Neuropsychology Review* vol. 3 (1992).
3. 2021 Alzheimer's disease facts and figures. *Alzheimer's Dement.* **17**, 327–406 (2021).
4. Thal, D. R., Walter, J., Saido, T. C. & Fändrich, M. Neuropathology and biochemistry of Aâ and its aggregates in Alzheimer's disease. *Acta Neuropathol.* **129**, 167–182 (2015).
5. Association, A. 2021 Alzheimer's disease facts and figures: Race , Ethnicity and Alzheimer's in America. *Race, Ethn. Alzheimer's Am.* **13**, 1–104 (2021).
6. Lin, L. Commonality between Diabetes and Alzheimer's Disease and a New Strategy for the Therapy. *Clin. Med. Pathol.* 1, CPath.S667 (2008).
7. Peila, R., Rodriguez, B. L. & Launer, L. J. *Type 2 Diabetes, APOE Gene, and the Risk for Dementia and Related Pathologies The Honolulu-Asia Aging Study. Diabetes*, **51** http://diabetesjournals.org/diabetes/article-pdf/51/4/1256/372544/db0402001256.pdf (2002).
8. Verdile, G., Fuller, S. J. & Martins, R. N. The role of type 2 diabetes in neurodegeneration. *Neurobiol. Dis.* **84**, 22–38 (2015).
9. Chornenkyy, Y., Wang, W. X., Wei, A. & Nelson, P. T. Alzheimer's disease and type 2 diabetes mellitus are distinct diseases with potential overlapping metabolic dysfunction upstream of observed cognitive decline. *Brain Pathology*, **29** (2019).
10. Sakr, H. F. Effect of sitagliptin on the working memory and reference memory in type 2 diabetic sprague-dawley rats: Possible role of adiponectin receptors 1. *J. Physiol. Pharmacol.* **64**, 613–623 (2013).
11. Kosaraju, J. *et al.* Vildagliptin: An anti-diabetes agent ameliorates cognitive deficits and pathology observed in streptozotocin-induced Alzheimer's disease. *J. Pharm. Pharmacol.* **65**, 1773–1784 (2013).
12. Athauda, D. & Foltynie, T. The glucagon-like peptide 1 (GLP) receptor as a therapeutic target in Parkinson's disease: Mechanisms of action. *Drug Discov. Today* **21**, 802–818 (2016).
13. Abdelsalam, R. M. & Safar, M. M. Neuroprotective effects of vildagliptin in rat rotenone Parkinson's disease model: Role of RAGE-NFêB and Nrf2-antioxidant signaling pathways. *J. Neurochem.* **133**, 700–707 (2015).
14. Bae, C. S. & Song, J. The role of glucagon-like peptide 1 (GLP1) in type 3 diabetes: GLP-1 controls insulin resistance, neuroinflammation and neurogenesis in the brain. *Int. J. Mol. Sci.* **18**, 16–18 (2017).
15. Chakrabarty Mrinmoy, Bhat Priyanka, Kumari Sweta, D'souza Avin, Bairy K. L., Chaturvedi Abhishek. Natarajan Archana, Rao K.G. Mohandas, K. S. Cortico-hippocampal salvage in chronic aluminium induced neurodegeneration by mrinmoy.pdf. 161–171 (2012).
16. Thippeswamy, A. H. *et al.* Evaluation of Bacopa monniera for its Synergistic Activity with Rivastigmine in Reversing Aluminum- Induced Memory Loss and Learning Deficit in Rats. **6**, 208–213 (2013).
17. Igwenagu, E., Igbokwe, I. O. & Egbe-Nwiyi, T. N. Fasting hyperglycaemia, glucose intolerance and pancreatic islet necrosis in albino rats associated with subchronic oral aluminium chloride exposure. *Comp. Clin. Path.* **29**, 75–81 (2020).
18. Buraimoh, A. A., Ojo, S. A., Hambolu, J. O. & Adebisi, S. S. Effects of Oral Administration of Aluminium Chloride on the Histology of the Hippocampus of Wistar Rats. *Curr. Res. J. Biol. Sci.* **3**, 509–515 (2011).
19. Kawahara, M. & Kato-Negishi, M. Link between aluminum and the pathogenesis of Alzheimer's disease: The integration of the aluminum and amyloid cascade hypotheses. *Int. J. Alzheimers. Dis.* 2011, (2011).
20. Vojdani, A. toxics Elevated IgG Antibody to Aluminum Bound to Human Serum Albumin in Patients with Crohn's, Celiac and Alzheimer's Disease. (2021) doi:10.3390/toxics9090212.
21. Ayush Kumar, Sanchari Basu Mallik, Samita Rijal, Nilanjan Changdar, Jayesh Mudgal, R. R. S. Dietary Oils Ameliorate Aluminum Chloride-Induced Memory Deficit in Wistar Rats. *Pharmacogn. Mag.* **13** (Suppl, 179–188 (2017).
22. Praveenkumar, S. E. *et al.* Amelioration of aluminium chloride (AlCl3) induced neurotoxicity by combination of rivastigmine and memantine with artesunate in Albino Wistar rats. *Biomed. Pharmacol. J.* **12**, 703–711 (2019).
23. Ahmed, H. H., Salem, A. M., Sabry, G. M., Husein, A. A. & Kotob, S. E. New insights in the horizon for the treatment of Alzheimer's Disease: A proposal based on experimental study. *Der Pharm. Lett.* **7**, 165–182 (2015).

24. Abdel-Wahab, W. M. AlCl3-Induced Toxicity and Oxidative Stress in Liver of Male Rats: Protection by Melatoni. *Life Sci. J.* **9**, (2012).

25. Kalaiselvi, A., Reddy, G. A. & Ramalingam, V. Ameliorating Effect of Ginger Extract (Z ingiber officinale Roscoe) on Liver Marker Enzymes , Lipid Profile in Aluminium chloride Induced Male Rats. *Int. J. Pharm. Sci. Drug Res.* **7**, 52–58 (2015).

26. N.R. Naidu, S. Bhat, D. U. Effect of Long Term Administration of Aluminium Chloride on. *Int. J. Pharm. Biol. Sci.* **3**, 616–622 (2013).

27. Kumar, A., Prakash, A. & Dogra, S. Neuroprotective effect of carvedilol against aluminium induced toxicity: Possible behavioral and biochemical alterations in rats. *Pharmacol. Reports* **63**, 915–923 (2011).

28. Konda, V. *et al.* Effect of aluminum chloride on blood glucose level and lipid profile in normal, diabetic and treated diabetic rats. *Indian J. Pharmacol.* **49**, 357–365 (2017).

29. Nivsarkar, M. *et al.* Reduction in Aluminum Induced Oxidative Stress by Meloxicam in Rat Brain. **10**, 151–155 (2006).

30. Yassin, N. A. Z. *et al.* Effect of Boswellia serrata on Alzheimer ' s disease induced in rats. **8**, 1–11 (2013).

31. Chakrabarty, M. *et al.* Cortico hippocampal salvage in chronic aluminium induced neurodegeneration by Celastrus paniculatus seed oil/ : Neurobehavioural , biochemical , histological study. **3**, 161–171 (2012).

32. Fernandes, J. *et al.* N-acetyl-L-tryptophan, a substance-P receptor antagonist attenuates aluminum-induced spatial memory deficit in rats. *Toxicol. Mech. Methods* **28**, 328–334 (2018).

33. Ellman L. George, Courtney, K. D. & Francisco, S. A new and rapid colorimetric of acetylcholinesterase determination. *Biochem. J.* **7**, 88–95 (1961).

34. Hossain, M., Chetana, M. & Uma Devi, P. Late effect of prenatal irradiation on the hippocampal histology and brain weight in adult mice. *Int. J. Dev. Neurosci.* **23**, 307–313 (2005).

35. Madhyastha, S., Somayaji, S. N., Rao, M. S., Nalini, K. & Bairy, K. L. Hippocampal brain amines in methotrexate-induced learning and memory deficit. *Can. J. Physiol. Pharmacol.* **80**, 1076–1084 (2002).

36. Suzanne M. de la Monte. Insulin resistance and Alzheimer's disease. *BMB Rep.* **42**, 475–481 (2009).

37. Nguyen, T. T. *et al.* Type 3 Diabetes and Its Role Implications in Alzheimer's Disease. *Int. J. Mol. Sci.* **21**, (2020).

38. Whitehouse, P. J. *et al.* Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. *Science (80-.).* **215**, 1237–1239 (1982).

39. Whitehouse, P. J., Price, D. L., Clark, A. W., Coyle, J. T. & DeLong, M. R. Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann. Neurol.* **10**, 122–126 (1981).

40. Dash Devasrita , Chogtu Bharti, K. B. L. Metformin Attenuates Cognitive Deficits in Experimentally Induced Alzheimer's Disease. *J. Glob. Pharma Technol.* **1**, 8–12 (2017).

41. Tayebati, S. K., Di Tullio, M. A. & Amenta, F. Effect of treatment with the cholinesterase inhibitor rivastigmine on vesicular acetylcholine transporter and choline acetyltransferase in rat brain. *Clin. Exp. Hypertens.* **26**, 363–373 (2004).

42. Sharma, K. Cholinesterase inhibitors as Alzheimer's therapeutics (Review). *Mol. Med. Rep.* **20**, 1479–1487 (2019).

43. Bailey, J. A., Ray, B., Greig, N. H. & Lahiri, D. K. Rivastigmine lowers Aâ and increases sAPPá levels, which parallel elevated synaptic markers and metabolic activity in degenerating primary rat neurons. *PLoS One* **6**, (2011).

44. Ali, M. A., El-Abhar, H. S., Kamel, M. A. & Attia, A. S. Antidiabetic Effect of Galantamine: Novel Effect for a Known Centrally Acting Drug. (2015) doi:10.1371/journal.pone.0134648.

45. Ali, M. A., El-Abhar, H. S., Kamel, M. A. & Attia, A. S. Antidiabetic effect of galantamine: Novel effect for a known centrally acting drug. *PLoS ONE* **10** (2015).

46. Xiao, L. *et al.* Neurotrophic factor-á1, a novel tropin is critical for the prevention of stress-induced hippocampal CA3 cell death and cognitive dysfunction in mice: comparison to BDNF. *Transl. Psychiatry* **11**, (2021).

47. Salim, S. Minireviews Oxidative Stress and the Central Nervous System. *J. Pharmacol. Exp. Ther. J Pharmacol Exp Ther* **360**, 201–205 (2017).

48. Cherian SB,, Bairy KL, R. M. Effect of chronic prenatal restraint stress on hippocampal neuronal cell density in male and female wistar rats at weaning. *Int. J. Recent Sci. Res.* **8**, 17153–17158 (2017).

49. Adelodun, S. T. *et al.* Aluminium chloride-induced hippocampal damage: CA3 hippocampal subfield involvement and the neuroprotective role of Buchholzia coriacea ethanolic seed extract. *Phytomedicine Plus* **1:** 100104 (2021).

50. Barkur, R. R. & Bairy, L. K. Evaluation of passive avoidance learning and spatial memory in rats exposed to low levels of lead during specific periods of early brain development. *Int. J. Occup.*

Med. Environ. Health **28**, 533–544 (2015).

51. Chalichem, N. S. S., Gonugunta, C., Krishnamurthy, P. T. & Duraiswamy, B. DPP4 Inhibitors Can Be a Drug of Choice for Type 3 Diabetes: A Mini Review. *American Journal of Alzheimer's Disease and other Dementias* **32** (2017).

52. Rizzo, M. R. *et al*. Dipeptidyl peptidase-4 inhibitors have protective effect on cognitive impairment in aged diabetic patients with mild cognitive impairment. *Journals Gerontol. - Ser. A Biol. Sci. Med. Sci.* **69**, 1122–1131 (2014).

53. Perry, T. & Greig, N. Enhancing Central Nervous System Endogenous GLP-1 Receptor Pathways for Intervention in Alzheimers Disease. *Curr. Alzheimer Res.* **2**, 377–385 (2005).

54. During, M. J. *et al*. Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nat. Med.* **9**, 1173–1179 (2003).

55. Khalaf, S. S., Hafez, M. M., Mehanna, E. T., Mesbah, N. M. & Abo-Elmatty, D. M. Combined vildagliptin and memantine treatment downregulates expression of amyloid precursor protein, and total and phosphorylated tau in a rat model of combined Alzheimer's disease and type 2 diabetes. *Naunyn. Schmiedebergs. Arch. Pharmacol.* **392**, 685–695 (2019).

56. Jamshidnejad-Tosaramandani, T., Kashanian, S., Babaei, M., Al-Sabri, M. H. & Schiöth, H. B. The potential effect of insulin on ache and its interactions with rivastigmine in vitro. *Pharmaceuticals* **14**, 1–11 (2021).

57. Zina Kroner, D. The Relationship between Alzheimer's Disease and Diabetes: Type 3 Diabetes? *Altern. Med. Rev.* **14**, 373 (2009).

58. Montgomery, M. K. & Turner, N. Mitochondrial dysfunction and insulin resistance/ : an update. *Endocr. Connect.* **4**, R1–R15 (2015).

59. Xu, F. *et al*. Aluminum chloride caused liver dysfunction and mitochondrial energy metabolism disorder in rat. *J. Inorg. Biochem.* **174**, 55–62 (2017).

60. Moreira, P. I., Carvalho, C., Zhu, X., Smith, M. A. & Perry, G. Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. *Biochim. Biophys. Acta - Mol. Basis Dis.* **1802**, 2–10 (2010).

61. Pintana, H., Apaijai, N., Chattipakorn, N. & Chattipakorn, S. C. DPP-4 inhibitors improve cognition and brain mitochondrial function of insulin-resistant rats. *J. Endocrinol.* **218**, 1–11 (2013).

62. Yuriko *et al*. The dipeptidyl peptidase-4 inhibitor linagliptin ameliorates high-fat induced cognitive decline in tauopathy model mice. *Int. J. Mol. Sci.* **20**, (2019).

63. Perry, T. & Greig, N. A New Alzheimers Disease Interventive Strategy: GLP-1. *Curr. Drug Targets* **5**, 565–571 (2004).

64. Zhang, D. D. *et al*. Vildagliptin, a DPP4 inhibitor, alleviates diabetes-associated cognitive deficits by decreasing the levels of apoptosis-related proteins in the rat hippocampus. *Exp. Ther. Med.* **15**, 5100–5106 (2018).

65. Li, Y., Zheng, M., Sah, S. K., Mishra, A. & Singh, Y. Neuroprotective influence of sitagliptin against cisplatin-induced neurotoxicity, biochemical and behavioral alterations in Wistar rats. *Mol. Cell. Biochem.* **455**, (2019).

66. Pujadas, G. *et al*. The dipeptidyl peptidase-4 (DPP-4) inhibitor teneligliptin functions as antioxidant on human endothelial cells exposed to chronic hyperglycemia and metabolic high-glucose memory. *Endocrine* **56**, (2017).

67. Abbas, T., Faivre, E. & Hölscher, C. Impairment of synaptic plasticity and memory formation in GLP-1 receptor KO mice: Interaction between type 2 diabetes and Alzheimer's disease. *Behav. Brain Res.* **205**, 265–271 (2009).