

Vitamin D3 Attenuates Type 3 Diabetic-Associated Cognitive Deficits in Rats through Regulating Neurotrophins and Enhancing Cholinergic Transmission Pathway

Yahya Abdullah Al-Zahrani, Mai A. Alim A. Sattar¹, Sameer E. Al-Harhi¹, Ayed A. Alkatheeri, Yahya Mohammed Al-Zahrani²

Department of Pharmacy, East Jeddah Hospital, Ministry of Health, ¹Department of Medical Pharmacology, College of Medicine, King Abdul-Aziz University, Jeddah, ²Department of Pharmacy, Directorate of Public Health, Ministry of Health, Makkah, Saudi Arabia

Abstract

Objective: To examine the protective effect of Vitamin D3 against Type 3 diabetes-induced cognitive dysfunction in rats. **Materials and Methods:** Type 3 diabetes was induced by a high-fat diet plus streptozotocin in rats. Rats were divided into seven groups: negative control, positive control, Vitamin D3 groups (100, 500 and 1000 IU/kg/day), Vitamin D3 plus rivastigmine, and rivastigmine monotherapy. A radial arm maze test was used to assess cognitive function. Levels of acetylcholinesterase (AChE), dopamine (DA), nerve growth factor, neurotrophin-3 (NT-3), and glial cell line-derived neurotrophic factor (GDNF) in the hippocampus were estimated by the enzyme-linked immunosorbent assay kits. **Results:** Chronic treatment with Vitamin D3 significantly ($P < 0.05$) and dose dependently alleviated cognitive deficits, with enhancing cholinergic transmission pathway activity through attenuated hippocampal AChE and increased DA level ($P < 0.001$). Moreover, Vitamin D3 significantly increased ($P < 0.001$) neurotrophin levels as an underlying mechanism for the resulted improvement. **Conclusion:** Vitamin D3 plus rivastigmine (combined group) is better than Vitamin D (100 and 500 mg/kg/day) for improvement of AChE, DA, NT-3, and GDNF levels. Vitamin D (500 and 1000 IU/kg/day) was effective as a combined group in terms of the behavioral test.

Keywords: Acetylcholinesterase, cholecalciferol, cognitive dysfunction, dementia, neurotrophic factors, rivastigmine

INTRODUCTION

Alzheimer's disease (AD) is a noncurable progressive neurodegenerative disorder that leads to tremendous loss of neurons, cognitive dysfunction, and gradual deterioration of memory. The neuropathological features of AD are extracellular deposition of β -amyloid ($A\beta$) in the brain's parenchyma and intracellular agglomeration of hyperphosphorylated tau protein (τ).^[1]

The exact mechanisms underlying diabetes-associated cognitive deficits are still unknown. Several studies have been carried out to explain the underlying mechanisms through which diabetes mellitus (DM) contributes to cognitive dysfunction. The aggregation of neurofibrillary tangles, altered processing of amyloid precursor protein, disturbance of neurotransmitters, and oxidative stress is among the suggested mechanisms.^[2,3]

Acetylcholine (ACh) and dopamine (DA) are primary neurotransmitters that play a crucial role in integrating

functions of learning and memory in the hippocampus.^[4] The disturbance of cholinergic transmissions in the hippocampus and cortex has resulted in cerebral hypoperfusion and accumulation of amyloid-beta, which, in turn, have caused cognitive impairment.^[5]

Neurotrophins are a family of proteins that include nerve growth factor (NGF), neurotrophin-3 (NT-3), and glial cell line-derived neurotrophic factor (GDNF). Neurotrophins

Address for correspondence: Yahya Mohammed Al-Zahrani,
Department of Pharmacy, Directorate of Public Health, Ministry of Health,
Makkah, Saudi Arabia.
E-mail: yahya-alhopen@hotmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

Access this article online

Quick Response Code:



Website:
www.jpharmacol.com

DOI:
[10.4103/jpp.jpp_20_21](https://doi.org/10.4103/jpp.jpp_20_21)

How to cite this article: Al-Zahrani YA, Sattar MA, Al-Harhi SE, Alkatheeri AA, Al-Zahrani YM. Vitamin D3 attenuates type 3 diabetic-associated cognitive deficits in rats through regulating neurotrophins and enhancing cholinergic transmission pathway. J Pharmacol Pharmacother 2021;12:47-53.

Received: 12-02-2021

Revised: 06-04-2021

Accepted: 27-04-2021

Web Publication: 17-09-2021

are considered survival factors for the several classes of neurons.^[6,7] Spatial navigation is compromised with the decreased synthesis of neurotrophins.^[8] DM and its associated changes of neurotrophic factors in the brain as well as their role in the pathogenesis of diabetes in AD are not manifested.

Vitamin D supplementation in animal studies reduced the number of amyloid plaques, attenuation of inflammation, stimulated A β phagocytosis, clearance, and brain to blood efflux.^[9-11] In addition, Serum 1,25 dihydroxy vitamin D (1,25(OH)₂D₃) was suggested to alter cholinergic, dopaminergic, and noradrenergic neurotransmitter systems in the central nervous system.^[12] Vahlsing *et al.* transplanted NGF overexpressing cells into the brains of AD patients and managed to reduce the rate of cognitive decline by 36%.^[13] Replacement of neurotrophic factors, such as NGF, GDNF, and neurotrophins and stem cell-related approaches to the treatment of AD are still being investigated.^[14] Some studies reported that the Serum 1,25 dihydroxy vitamin D (1,25(OH)₂D₃) is correlated with neurotrophins regulation.^[15] However, its relation to memory dysfunction through the regulation of neurotrophic factors has not been elucidated.

The present study was designed to examine whether Vitamin D₃ has a protective effect against diabetes-induced cognitive dysfunction in rats through the regulation of neurotrophic factors and neurotransmitters.

MATERIALS AND METHODS

Animals

Eighty-four healthy male albino rats (6–9-week-old, 175–220 g) were procured from King Fahd Center for Medical Research, KAU. All procedures in this study were applied in compliance with ethical regulations approved by the research ethics committee of King Abdul-Aziz University (Approval number 488-17). Rats were housed under standard laboratory conditions (at an ambient temperature of 24°C–26°C and relative humidity of 50%–70%) with a 12 h light/dark cycle. All rats were fed with a regular diet and drinking water *ad libitum*.

Chemicals and reagents

Streptozotocin (STZ) and rivastigmine were purchased from Sigma Aldrich Chemical Company, (CO., Saint Louis, MO, USA) as a white powder. Cholecalciferol (Vitamin D₃ Oral drops 4500 IU/l, Novartis International AG, Basel, Switzerland) was used in this study. Rat ELISA kits for measurement of acetylcholinesterase (AChE), DA, NGF, NT-3, and GDNF were purchased from My Biosource, Inc. (Southern California, San Diego, USA).

Drugs doses and preparations

Citrate buffer (0.1M) was prepared by diluting 2.1 g citric acid with 2.94 g sodium citrate in 100 ml sterile water then, NaOH/HCl was added for adjusting the pH to 4.5, using a calibrated pH meter.^[16] STZ was freshly prepared immediately before the use by dissolving in 0.1 M sodium citrate buffer, pH 4.5,^[16] and

intraperitoneally injected at a dose of 40 mg/kg body weight.^[17] Rivastigmine was prepared daily by dissolving in sterile water and orally administered at a dose of 1 mg/kg/day.^[18,19] Three graded doses of Vitamin D₃ 100, 500 and 1000 IU/kg/day were used in this experiment based on previous studies.^[20,21] Vitamin D₃ was administered orally by gavage.

Model induction and experimental design

Rats were divided into the negative control group ($n = 12$) was fed with a regular diet, and the high fat diet (HFD) group ($n = 72$) was given HFD diet. Four weeks of dietary manipulation (HFD) resulted in insulin-resistant animals, and then, rats in the HFD group were intraperitoneally injected with STZ. Rats having fasting blood glucose >200 mg/dL, 72 h after T2D induction was considered diabetic rats and selected for this study.^[22] After diabetes induction, all 84 rats were grouped into seven groups: Group I: Negative control rats: Injected with citrate buffer (pH 4.5) (1 ml/kg, i. p). Diabetic rats were randomly divided into six groups comprising 12 rats each per group: Group II – nontreated DM rats – positive control; Group III – DM rats received oral 100 IU/kg of Vitamin D₃ once daily; Group IV – DM rats received oral 500 IU/kg of Vitamin D₃ once daily; Group V – DM rats received oral 1000 IU/kg of Vitamin D₃ once daily; Group VI (combined group) – DM rats received oral 500 IU/kg of Vitamin D₃ once daily plus rivastigmine 1 mg/kg/day; and Group VII – T3D rats received oral rivastigmine 1 mg/kg/day. The treatment period lasted for 16 weeks, and rats were kept feeding on their respective diet until the end of the study. To treat rats with accurate doses over the entire period of this study, doses of vitamin D₃ and rivastigmine were adjusted every 2 weeks according to the bodyweight changes.

Assessment of cognitive function by radial arm maze test

The radial arm maze (RAM) in the current study consisted of eight arms of 75 cm length, 12 cm width, and 14 cm height, and all arms have a similar radiating angle from the central platform (32 cm in diameter). To decrease the variability of each test, the maze was located at a fixed position throughout the whole experiment period. In our study, baited and unbaited arms were also fixed. The baited arms (with food) were the 1st, 3rd, 5th, and 7th arms, whereas unbaited arms (without food) were the 2nd, 4th, 6th, and 8th arms. All rats were trained for 5 days before the testing day, one session per day. In each test session, each rat was placed in the central starting area of the equipment facing toward the first arm. The baited arms are expected to visit by food-denied rats, and these rats subsequently register and retain the memory of each visited arm where food was present. Each rat was allowed to freely explore and consume food rewards for 5 min or until all food rewards of the four baited arms were eaten, whichever came first. The maze was then cleaned entirely with 70% alcohol before the next test session to avoid odors guidance of food from previous tests. On the testing day (6th day), latency to complete the whole task, reference memory error (RME), and working memory error (WME), were recorded. The RME was recorded when rat first entry or reentry into the never-baited

arms, while a WME was recorded when rat reentry into arms where the food reward had already been eaten arm is considered reference-WMEs.^[23]

Hippocampus preparation and biochemical analyses

One day after the completion of the behavioral experiment, rats were decapitated and hippocampi were immediately dissected, placed on ice, washed in cold 0.9% normal saline, and weighed. Rats hippocampi were subsequently homogenized in sodium phosphate buffer (pH 6.9) with a glass homogenizer on the ice and the homogenate was centrifuged at 5000 g. The clear supernatants obtained were used to estimate the hippocampal levels of AChE, DA, NGF, NT-3, and GDNF by quantification ELISA kits following the company's recommended protocol.^[24]

Statistical analysis

Statistical analysis was accomplished using the Statistical Package for the Social Sciences (SPSS) version 26.0 (SPSS

Inc., Chicago, IL, USA). The data were analyzed using one way analysis of variance followed by Tukey test for multiple comparisons. $P \leq 0.05$ was considered statistically significant.

RESULTS

Vitamin D3 reverses learning defects and corrects the T3D-induced spatial memory loss

Data obtained from the RAM test in Figure 1 demonstrated a significant decline in the acquisition of non-treated diabetic rats and Vitamin D3 treatment significantly improved it toward that in the negative control rats. The number of entries into unbaited arms (RME) of RAM test in diabetic rats treated by Vitamin D3 groups (500 and 1000 IU/kg/day), combined group, and rivastigmine monotherapy significantly decreased ($P < 0.05$), ($P < 0.01$), ($P < 0.01$), and ($P < 0.01$), respectively, as compared to nontreated diabetic rats and nonsignificant decrease ($P > 0.05$) in RME of Vitamin D3 group (100 IU/kg/day) was observed versus positive

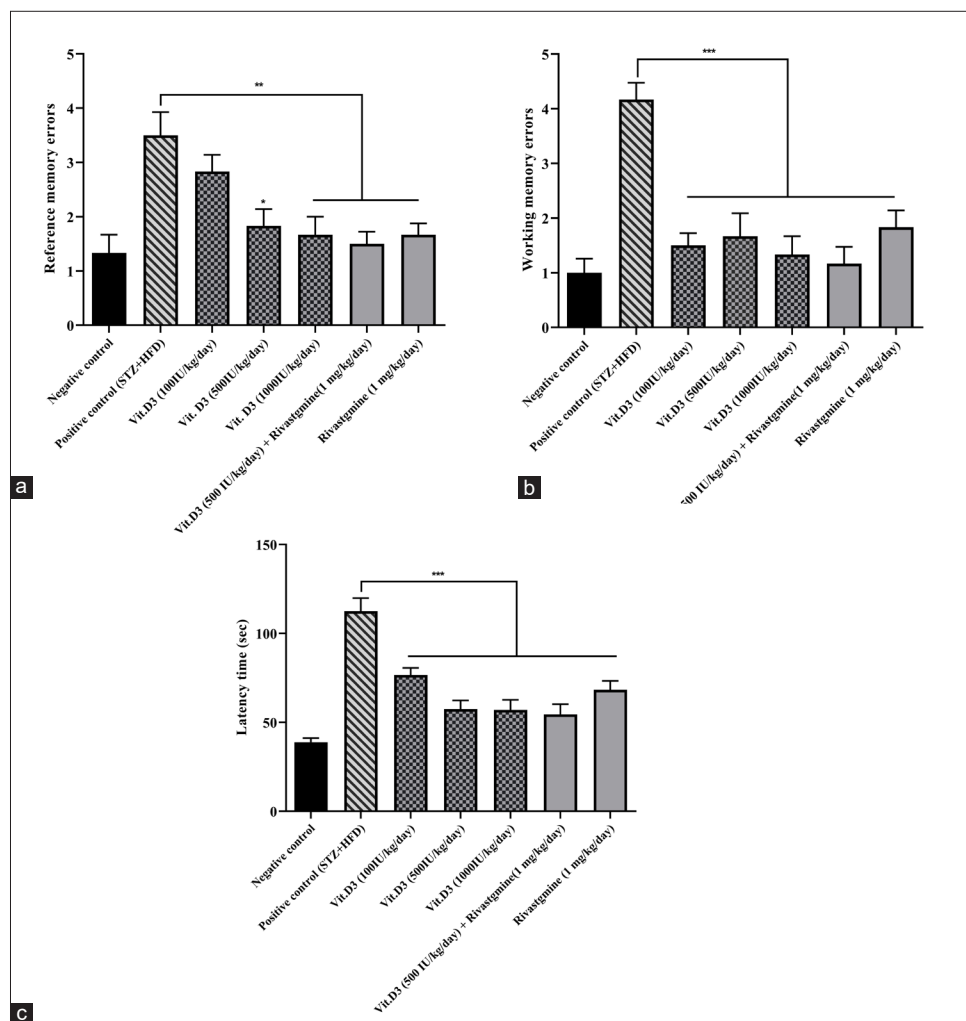


Figure 1: Effect of Vitamin D3 on (a) Reference memory error, (b) working memory error, and (c) latency time in the radial arm maze test of T3D-induced Alzheimer in rats. ** $P < 0.01$, *** $P < 0.001$ compared with the corresponding positive control group values; by one-way analysis of variance and Tukey honestly significant difference *post hoc* test. Data expressed as the mean $\pm$ standard error of the mean; $n = 12$ rats

control [Figure 1a]. In the meantime, we also observed that the number of reentries into the same arms (WME) and the time required to finish the task in the RAM (latency time) was significantly lower ($P < 0.001$) in all treated diabetic rats as compared to nontreated diabetic rats [Figure 1b and c]. Nevertheless, there was no significant difference ($P > 0.05$) in RMEs, WMEs, and latency time of RAM test in all treated experimental groups as compared with the rivastigmine group.

Type 3 diabetes enhances the activity of AChE and reduces DA levels in the hippocampus and Vitamin D3 reverses this effect.

Figure 2 revealed that the chronic administration of Vitamin D3 dose (1000 IU/kg/day), Vitamin D3 plus rivastigmine group, and rivastigmine monotherapy for 4 months was markedly reduced AChE level compared with nontreated diabetic rats ($P < 0.05$), ($P < 0.01$), and ($P < 0.01$), respectively, with mean values $\pm$ standard error of the mean (SEM) of $2.89 \text{ nmol/l} \pm 0.11$, $2.41 \text{ nmol/l} \pm 0.13$, and $2.47 \text{ nmol/l} \pm 0.20$, respectively. Furthermore, a significant decrease ($P < 0.05$) was observed in the AChE level of Vitamin D3 groups (100 and 500 IU/kg/day) as compared with the rivastigmine group [Figure 2a]. In the meantime, DA concentration was increased significantly in Vitamin D3 groups (100, 500, and 1000 IU/kg/day), Vitamin D3 plus rivastigmine group, and rivastigmine group compared with the positive control group ($P < 0.001$) with mean values $\pm$ SEM of $9.76 \text{ pg/ml} \pm 0.56$, $10.31 \text{ pg/ml} \pm 0.46$, $11.14 \text{ pg/ml} \pm 0.65$, $12.72 \text{ pg/ml} \pm 0.52$, and $10.08 \text{ pg/ml} \pm 0.43$, respectively. Furthermore, there was no significant difference ($P > 0.05$) in DA level between all Vitamin D3-treated groups compared with and rivastigmine group [Figure 2b].

Neurotrophic factors elevated with sustained use of Vitamin D3 in T3D-induced Alzheimer in rats

Figure 3 showed that NGF, NT-3, and GDNF levels in hippocampal tissue of nontreated diabetic rats were significantly reduced ($P < 0.001$, $P < 0.001$, and $P < 0.05$, respectively) as compared with nondiabetic healthy rats. We also observed that the administration of Vitamin D3 doses (100, 500, and 1000 IU/kg/day) and Vitamin D3 plus rivastigmine group for 16 weeks markedly elevated hippocampal NGF [$P < 0.001$; Figure 3a], NT-3 [$P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.001$, reps; Figure 3b] and GDNF [$P < 0.05$, $P < 0.05$, $P < 0.001$, and $P < 0.001$, respectively; Figure 3c] concentration as compared with nontreated diabetic rats. While a nonsignificant change ($P > 0.05$) in NGF, NT-3, and GDNF levels was seen in the rivastigmine group as compared to positive control. In compared to rivastigmine-treated group, Vitamin D3 doses (100, 500, and 1000 IU/kg/day) and Vitamin D3 plus rivastigmine group exhibited a significant increase of NGF level ($P < 0.01$, $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively). In the meantime, NT-3 hippocampal level was significantly increased in Vitamin D3 groups (100, 500, and 1000 IU/kg/day) and Vitamin D3 plus rivastigmine group ($P < 0.01$, $P < 0.05$, $P < 0.001$, and $P < 0.001$, respectively) as compared to the rivastigmine group. Although GDNF level in Vitamin D3 group (1000 IU/kg/day) and Vitamin D3 plus rivastigmine group was found to be higher than rivastigmine group, this change was significant ($P < 0.001$).

DISCUSSION

The chief findings of this study were as follows: (1) HFD and IP injection of STZ produced significant deficits of learning and memory, increased AChE activity, while reduced DA, NGF, NT-3, and GDNF levels in the hippocampus, (2)

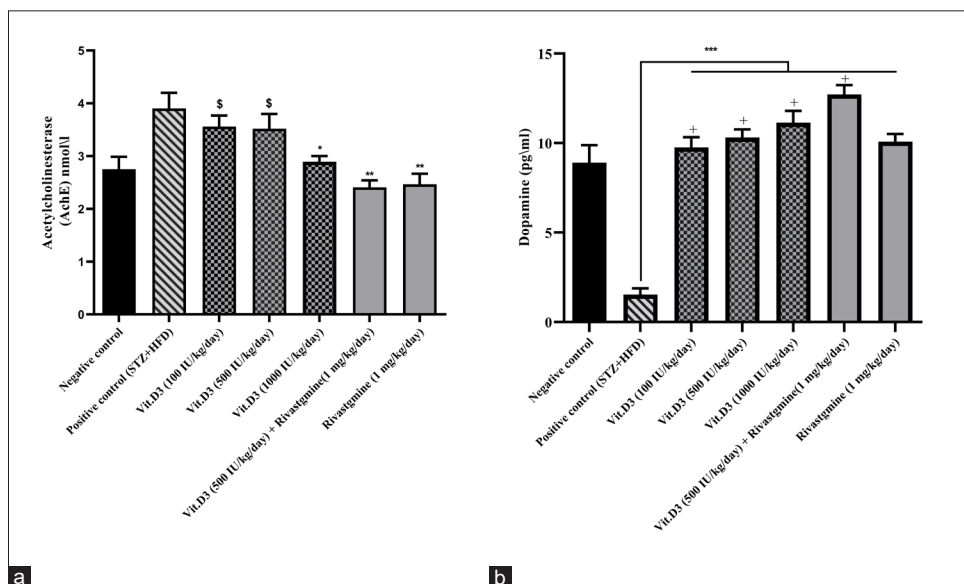


Figure 2: Effect of Vitamin D3 on (a) acetylcholinesterase activity and (b) Dopamine level in the hippocampal brain tissue of T3D-induced Alzheimer rats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the corresponding positive control group values; § $P < 0.05$ compared with the corresponding rivastigmine group values; by the one-way analysis of variance and Tukey honestly significant difference *post hoc* test. Data expressed as the mean $\pm$ standard error of the mean; $n = 12$ rats

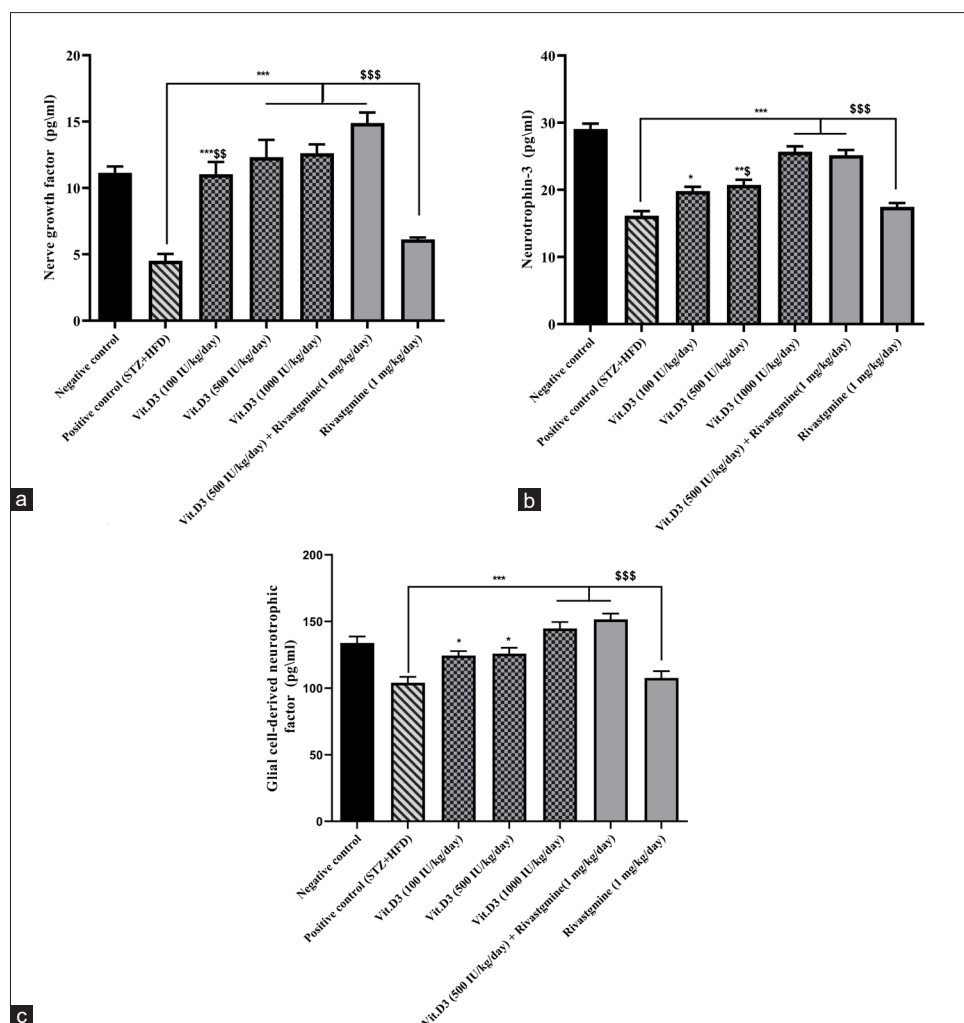


Figure 3: Effect of Vitamin D3 on (a) nerve growth factor, (b) neurotrophin-3 (c) glial cell line-derived neurotrophic factor in the hippocampal brain tissue of T3D-induced Alzheimer rats. * $P < 0.05$, *** $P < 0.01$, **** $P < 0.001$ compared with the corresponding positive control group values; \$ $P < 0.05$, \$\$ $P < 0.01$, \$\$\$ $P < 0.001$ compared with the corresponding rivastigmine group values; by one-way analysis of variance and Tukey honestly significant difference *post hoc* test. Data expressed as the mean $\pm$ standard error of the mean; $n = 12$ rats

Vitamin D3 reversed the STZ-induced cognitive dysfunction significantly and enhanced hippocampal DA, NGF, NT-3, and GDNF levels, (3) Vitamin D3 also reduced the level of AChE.

The present study showed that the administration of Vitamin D3 produced a significant enhancement of cognitive function in RAM, as apparent by the significantly reduced RME, WME, and latency in Vitamin D3 treated versus untreated T3D rats. This result represents the value of the prophylactic use of Vitamin D3 in the improvement of learning ability and memory performance. The results of the current study go hand in hand with previous findings that also showed an elevated maze performance of the animals after 1,25(OH) 2D3 supplementation compared with a nontreated group.^[25]

ACh and DA play a crucial role in facilitating learning and memory, and therefore, the disturbance in the release of these neurotransmitters will result in memory impairment.^[26]

The results of the present work regarding AChE increase and DA decrease in diabetic rats were in agreement with those of previous results, which revealed that chronic hyperglycemia in diabetes is reported to diminish dopaminergic functions.^[27] Moreover, Huang *et al.* stated that chronic DM produced a significant increase in AChE level in the diabetic-induced Alzheimer animal model.^[28]

Reduced AChE activity ($P < 0.001$) and enhanced DA level ($P < 0.001$) in the hippocampus of diabetic rats in the present study after Vitamin D administration were also established in previous studies; Kumar *et al.* demonstrated the neuroprotective role of Vitamin D in the cerebral cortex by normalizing the altered cholinergic synaptic transmission in streptozocin-induced diabetic rats.^[12]

Neurotrophins such as NGF, NT-3, and GDNF are essential for the maturity, maintenance, and survival of particular neurons and also have been associated with controlling and

coordinating the normal functioning of the hippocampal pathway, which is required in learning ability and memory capacity. When neurotrophin synthesis is decreased, spatial navigation is compromised. NGF is vital in the plasticity and survival of forebrain cholinergic neurons, which are memory-related.^[29] GDNF is a critical growth factor for the growth, survival, and maintenance of dopaminergic neurons.^[30] Depletion of NGF and GDNF seems to be linked with the pathophysiology of AD. NT-3, a protein found in the hippocampus and neocortex, reduces the toxicity of neurons by amyloid-beta through limiting caspase-8, caspase-9, and caspase-3 cleavage. Moreover, NT-3 produces the upregulation of neuronal apoptosis inhibitory protein-1 expression in neurons that promote the inhibition of A β -induced neuronal apoptosis.^[31]

To our knowledge, there is no published study to assess neurotrophic factors changes associated with diabetic-induced Alzheimer's animal model. Our results indicate that diabetes-induced downregulation of NGF, NT-3, and GDNF in nontreated diabetic rats may be contributing to cognitive dysfunction.

Interestingly, in the current study, Vitamin D3 administration was found to be disease modifying agent in AD as it significantly increased ($P < 0.001$) the NGF, NT-3, and GDNF levels in the hippocampus of all Vitamin D3 treated rats compared with the nontreated diabetic rats. These findings are consistent with previous work which revealed that the synthesis of NGF, NT3, and GDNF was upregulated by 1,25-(OH)2D3, whereas neurotrophin 4 was downregulated.^[32] Similarly, the stimulation of neurotrophin production by 1,25-(OH)2D3 was correlated with a neuroprotective effect.^[33] Moreover, in animal models, treatment with 1, 25 (OH)2D3 increased GDNF concentrations and reduced oxidative stress in Parkinson's disease.^[34]

In the current work, rivastigmine did not affect neurotrophic factors but its effect potentiated by the addition of Vitamin D. These results differ from previous studies, which concluded that AChE inhibitors increase neurotrophic factors levels.^[35-38] Regarding the contradictory results, it must be noted that the neurotrophic factor levels in the previous studies were measured immediately (4-24 h) after the administration of AChE inhibitors. Our findings demonstrated that steady AChE inhibition caused a nonsignificant change in the levels of neurotrophic factors in the hippocampus. In accordance with our result, Autio *et al.* 2011 stated that chronic treatment with galantamine (3 mg/kg, i. p., 14 days) did not induce changes in hippocampal NGF and BDNF synthesis or protein levels.^[39] Therefore, AChE inhibitors may have short-term effects on brain neurotrophin levels. Another explanation of the results would be the antioxidant activity of Vitamin D as we reported in our previous study while we failed to detect this effect in the rivastigmine group.^[40]

CONCLUSION

This study was the first of its kind that focused on the effects

of vitamin D3 treatment on T3 DM induced cognitive function. The novelty of this study comes from the data that diabetes triggers a neurodegeneration mechanism not only by producing the A β deposition but also suppressing the NGF, NT-3, and GDNF. Vitamin D protected neurons by (1) reducing AChE level and (2) upregulating DA, NGF, NT-3, and GDNF.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Kumar A, Singh A, Ekavali. A review on Alzheimer's disease pathophysiology and its management: An update. *Pharmacol Rep* 2015;67:195-203.
- de la Torre JC. Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. *Lancet Neurol* 2004;3:184-90.
- Marrow B, Secreted S, and Protect C. Diabetes exacerbates amyloid and neurovascular pathology in aging-accelerated mice. *Aging Cell* 2014; 71:3831-3840.
- Reis HJ, Guatimosim C, Paquet M, Santos M, Ribeiro FM, Kummer A, *et al.* Neuro-transmitters in the central nervous system and their implication in learning and memory processes. *Curr Med Chem* 2009;16:796-840.
- Xiong L, Zhang JJ, Sun D, Liu H. Therapeutic benefit of Yangxue Qingnao Granule on cognitive impairment induced by chronic cerebral hypoperfusion in rats. *Chin J Integr Med* 2011;17:134-40.
- Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci* 2001;24:677-736.
- Skaper SD. The neurotrophin family of neurotrophic factors: An overview. *Methods Mol Biol* 2012;846:1-2.
- Sampaio TB, Savall AS, Gutierrez ME, Pinton S. Neurotrophic factors in Alzheimer's and parkinson's diseases: Implications for pathogenesis and therapy. *Neural Regen Res* 2017;12:549-57.
- Ji C, Song C, Zuo P. The mechanism of memory impairment induced by A β chronic administration involves imbalance between cytokines and neurotrophins in the rat hippocampus. *Curr Alzheimer Res* 2011;8:410-20.
- Dursun E, Gezen-Ak D, Yilmazer S. A novel perspective for Alzheimer's disease: Vitamin D receptor suppression by amyloid- β and preventing the amyloid- β induced alterations by vitamin D in cortical neurons. *J Alzheimers Dis* 2011;23:207-19.
- Yu J, Gattoni-Celli M, Zhu H, Bhat NR, Sambamurti K, Gattoni-Celli S, *et al.* Vitamin D3-enriched diet correlates with a decrease of amyloid plaques in the brain of A β PP transgenic mice. *J Alzheimers Dis* 2011;25:295-307.
- Kumar PT, Antony S, Nandhu MS, Sadanandan J, Najil G, Paulose CS. Vitamin D3 restores altered cholinergic and insulin receptor expression in the cerebral cortex and muscarinic M3 receptor expression in pancreatic islets of streptozotocin induced diabetic rats. *J Nutr Biochem* 2011;22:418-25.
- Vahlsing HL, Hansen L, Bakay R, Gall C, U HS, Conner J, *et al.* A phase I clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nat Med* 2005; 11:551-555.
- Sánchez-López E, Abad S, García ML, Beas-Zarate C, Olloquequi J, Ettcheto M, *et al.* Current research therapeutic strategies for Alzheimer's disease treatment. *Neural Plast* 2016;2016:1-5.
- Garcion E, Wion-Barbot N, Montero-Menei CN, Berger F, Wion D. New clues about vitamin D functions in the nervous system. *Trends Endocrinol Metab* 2002;13:100-5.
- Ghasemi A, Khalifi S, Jedi S. Streptozotocin-nicotinamide-induced rat model of type 2 diabetes (review). *Acta Physiol Hung* 2014;101:408-20.
- Gheibi S, Kashfi K, Ghasemi A. A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. *Biomed Pharmacother* 2017;95:605-13.

18. Bihagi SW, Sharma M, Singh AP, Tiwari M. Neuroprotective role of *Convolvulus pluricaulis* on aluminium induced neurotoxicity in rat brain. *J Ethnopharmacol* 2009;124:409-15.
19. Nampoothiri M, John J, Kumar N, Mudgal J, Nampurath GK, Chamallamudi MR. Modulatory role of simvastatin against aluminium chloride-induced behavioural and biochemical changes in rats. *Behav Neurol* 2015;2015:210169.
20. Alrefaie Z, Alhayani A. Vitamin D₃ improves decline in cognitive function and cholinergic transmission in prefrontal cortex of streptozotocin-induced diabetic rats. *Behav Brain Res* 2015;287:156-62.
21. Salum E, Kampus P, Zilmer M, Eha J, Butlin M, Avolio AP, *et al.* Effect of vitamin D on aortic remodeling in streptozotocin-induced diabetes. *Cardiovasc Diabetol* 2012;11:58.
22. Mahajan S, Chauhan P, Mishra M, Yadav D, Debnath M, Prasad G. Antidiabetic potential of eugenia jambolana ethanolic seed extract: Effect on antihyperlipidemic and antioxidant in experimental streptozotocin-induced diabetic rats. *Adv Complement Alt Med* 2018;2:1-9.
23. Sahak MK, Mohamed AM, Hashim NH, Hasan Adli DS. *Nigella sativa* oil enhances the spatial working memory performance of rats on a radial arm maze. *Evid Based Complement Alternat Med* 2013;2013:1-5.
24. Engvall E, Perlmann P. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry* 1971;8:871-4.
25. Taghizadeh M, Talaie SA, Salami M. Vitamin D deficiency impairs spatial learning in adult rats. *Iran Biomed J* 2013;17:42-8.
26. Hasselmo ME. The role of acetylcholine in learning and memory. *Curr Opin Neurobiol* 2006;16:710-5.
27. Vallone D, Picetti R, Borrelli E. Structure and function of dopamine receptors. *Neurosci Biobehav Rev* 2000;24:125-32.
28. Huang X, Wang F, Chen W, Chen Y, Wang N, von Maltzan K. Possible link between the cognitive dysfunction associated with diabetes mellitus and the neurotoxicity of methylglyoxal. *Brain Res* 2012;1469:82-91.
29. Korsching S, Auburger G, Heumann R, Scott J, Thoenen H. Levels of nerve growth factor and its mRNA in the central nervous system of the rat correlate with cholinergic innervation. *EMBO J* 1985;4:1389-93.
30. Deister C, Schmidt CE. Optimizing neurotrophic factor combinations for neurite outgrowth. *J Neural Eng* 2006;3:172-9.
31. Lesné S, Gabriel C, Nelson DA, White E, Mackenzie ET, Vivien D, *et al.* Akt-dependent expression of NAIP-1 protects neurons against amyloid- β toxicity. *J Biol Chem* 2005;280:24941-7.
32. Brown J, Bianco JJ, McGrath JJ, Eyles DW. 1,25-dihydroxyvitamin D₃ induces nerve growth factor, promotes neurite outgrowth and inhibits mitosis in embryonic rat hippocampal neurons. *Neurosci Lett* 2003;343:139-43.
33. Hayashi T, Morales M, Su TP, Wang Y, Lin SZ, Chiang YH, *et al.* Vitamin D₃ attenuates cortical infarction induced by middle cerebral arterial ligation in rats. *Neuropharmacology* 2002;39:873-80.
34. Wang JY, Wu JN, Cherg TL, Hoffer BJ, Chen HH, Borlongan CV, *et al.* Vitamin D(3) attenuates 6-hydroxydopamine-induced neurotoxicity in rats. *Brain Res* 2001;904:67-75.
35. Da Penha Berzaghi M, Cooper J, Castren E, Zafra F, Sofroniew M, Thoenen H, *et al.* Cholinergic regulation of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) but not neurotrophin-3 (NT-3) mRNA levels in the developing rat hippocampus. *J Neurosci* 1993;13:3818-26.
36. Betancourt AM, Filipov NM, Carr RL. Alteration of neurotrophins in the hippocampus and cerebral cortex of young rats exposed to chlorpyrifos and methyl parathion. *Toxicol Sci* 2007;100:445-55.
37. French SJ, Humby T, Horner CH, Sofroniew MV, Rattray M. Hippocampal neurotrophin and trk receptor mRNA levels are altered by local administration of nicotine, carbachol and pilocarpine. *Brain Res Mol Brain Res* 1999;67:124-36.
38. Tang LL, Wang R, Tang XC. Effects of huperzine A on secretion of nerve growth factor in cultured rat cortical astrocytes and neurite outgrowth in rat PC12 cells. *Acta Pharmacol Sin* 2005;26:673-8.
39. Autio H, Mätlik K, Rantamäki T, Lindemann L, Hoener MC, Chao M, *et al.* Acetylcholinesterase inhibitors rapidly activate Trk neurotrophin receptors in the mouse hippocampus. *Neuropharmacology* 2011;61:1291-6.
40. Zahrani YA, Sattar MA, Alharthi SE, Alkreathy HM. Neuroprotective role of vitamin D₃ against insulin resistance and diabetic induced memory dysfunction in rats. *Int J Pharmacol* 2019;15:724-30.