Original Research Article

Neuroprotective effect of different doses of VitamineD3 in diabetic-induced Alzheimer rat model.

ABSTRACT

Background: Many studies revealed that diabetes is an independent risk factor for developing cognitive dysfunction, and Alzheimer. During diabetes, overexpression of nitric oxide, tumor necrosis factor, interleukin-6 and interleukin-1 beta leading to Aβ accumulation and neuronal death.

Aim: To examine the neuroprotective effect of different doses of vitamin D3 against diabetic-induced cognitive dysfunction in rats. Moreover, possible underlying mechanisms were also investigated.

Method: High-fat diet plus streptozotocin were used to induce diabetes in Westar rats. We sub-grouped the diabetic rats into six subgroups, positive control, vitamin D3 groups (100,500 and 1000 IU/kg/day), vitamin D3 plus rivastigmine, and rivastigmine monotherapy. After the induction of diabetes, we started treatment for sixteen months. Morris water maze test was used to evaluate cognitive function, followed by estimation of beta-amyloid-42, inducible nitric oxide synthase, nitric oxide, tumor necrosis factor, interleukin-6, and interleukin-1β levels in the hippocampus by ELIZA kits.

Results: Vitamin D3 treatment significantly ($p<0.05$) and dose-dependently mitigated cognitive deficits observed in Morris water maze test, with significant, suppresses in beta-amyloid-42 and nitric oxide synthase pathway via attenuated hippocampal inducible nitric oxide synthase and nitric oxide overproduction ($p<0.05$).Moreover, vitamin D3 decreased inflammation state of diabetic rats brains by significantly lowered ($p<0.05$) pro-inflammatory cytokines levels including, tumor necrosis factor, interleukin-6 and interleukin-1*β* as an underlying mechanism for the resulted improvement.

Conclusion: The results of this research suggest that upregulation of nitric oxide synthase pathway along with the increase in pro-inflammatory cytokines is critically involved in cognitive dysfunction associated with diabetes. VitaminD3 can ameliorate these effects and has a promising neuroprotective effect in diabetic-induced cognitive dysfunction.

Keywords: VitaminD3; Alzheimer; diabetes; nitric oxide; pro-inflammatory cytokines; beta-amyloid-42.

1. INTRODUCTION

Alzheimer disease (AD) is a chronic neurodegenerative disease featured by progressive loss of memory capacity and cognitive function required daily for activities performance. The neuropathological characteristics of AD are deposition of β-amyloid as senile plaque, and aggregation of tau protein to form neurofibrillary tangles[1] There are no accurate statistics on the prevalence of AD in Saudi Arabia. Fifty thousand patients in the Kingdom living with AD, most of them are women[2].

Many studies related to diabetes mellitus (DM) have revealed that DM is an independent risk factor for developing cognitive dysfunction, and dementia [3]. Multiple studies have been done trying to assess the underlying mechanisms by which DM participates to cognitive dysfunction. Accumulation of Amyloid β, aggregation of tau protein, oxidative stress, neurotransmitters disturbance, and inflammatory response are among the suggested mechanisms[4].

Chronic inflammatory processes play a crucial role in the AD progression. It has been reported that elevated proinflammatory cytokines levels, including interleukin 6 (IL-6), interleukin 1β (IL-1β) and tumor necrosis factor alpha (TNF-α), may diminish phagocytosis of Aβ in AD patients brains[5]. Also, inducible nitric oxide synthase (iNOS) influences the production of nitric oxide (NO) and is another main Aβ-induced constituent that contributes to the immune response. Immunoreactivity of iNOS was detected in the neurons and astrocytes of patients with AD[6] [7]. Aβ-mediated iNOS induction was proven to be caused by an increased level of TNF and IL1β in astrocytes and microglia[8]. iNOS and Aβ were observed to induce the accumulation of each other [9]. Furthermore, a low level of iNOS was reported to significantly lower Aβ accumulation, suppress phosphorylated tau aggregations, decrease protein tyrosine nitration and protect against gliosis[9].

Besides the crucial role of vitamin D3 on bone health, results from recent studies provide persuasive evidence on the function of vitamin D3 in other biological processes in different tissues, including the nervous system [10]. Moreover, clinical data have reported that high serum levels of 25-hydroxyvitamin D was strongly correlated with enhanced cognitive function performance and low vitamin D level was observed in Alzheimer patients [11].

Administration of 1,25 (OH) D to experimental rats was found to produce a change in the inflammatory processes and burden of amyloid β. More precisely, the elevation in proinflammatory cytokine Il-1 and the reduction of anti-inflammatory cytokine Il-10, observed in rats, is abolished after subcutaneously injected of vitamin D [12]. Also, a diet with low vitamin D enhanced oxidative stress through the transcription of iNOS[13].

The present study was designed to examine whether vitamin D3 has a protective role against diabetic - induced cognitive dysfunction in rats. Moreover, possible underlying mechanisms were also investigated.

2. MATERIAL AND METHODS

2.1 Experimental location and duration:

Experimental studies were carried out at King Fahd Center for Medical Research (KFCMR), King Abdul Aziz University (KAU), Jeddah, Saudi Arabia from July, 1 – December, 15 2018.

2.2 Ethical statement:

All procedures applied according to the ethical guidelines of the medical ethics committee of the King Abdul-Aziz University (KAU). The research ethics committee approved the animal protocol with Approval number 488-17.

2.3 Chemicals and reagents:

Streptozotocin and rivastigmine were obtained from Sigma Aldrich, (CO., Saint Louis, MO, USA) as a white powder. Oral drops of cholecalciferol (VitaminD3 4500 IU/mL, Novartis International AG, Basel, Switzerland) was used in this study. Rat ELISA kits for estimation of Beta-Amyloid peptide (Aβ), inducible nitric oxide synthase (iNOS), nitric oxide(NO), tumor necrosis factor(TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β) were bought from MyBiosource, Inc. (Southern California, San Diego (USA).

2.4 Drugs doses and preparations:

The citrate buffer (0.1M) was prepared by diluting of 2.1 g citric acid with 2.94 g sodium citrate in 100 ml sterile water. For adjustment of the pH to 4.5, concentrated NaOH/HCl was added with using a calibrated pH meter. Streptozotocin (STZ) was freshly prepared before use within 10 minutes by dissolving in 0. 1 M sodium citrate buffer and used at a dose of 40 mg/kg body weight [14]. To decrease the degradation of STZ by light, the solution prepared in 20 ml glass baker covered with aluminum foil. Rivastigmine was prepared daily in sterile water and orally administered by gavage at a dose of 1 mg/kg/day. The dose selection was based on the previous literature report [15]. Vitamin D3 given by oral gavage, three graded doses of vitamin D3 100,500 and 1000 IU/kg/day were selected based on previous studies[16].

2.5 Animal and housing:

Eighty-four male albino rats, aged 6-9 weeks of average weight± SD (208 g ± 18.63) were purchased from the experimental animal unit of King Fahd Medical Research Center (KFMC), KAU. Rats were housed in standard animal laboratory conditions; temperature ranged between 24° C - 26° C, relative humidity was between 50 % 70% and a 12 h light/dark cycle. All animals were allowed to one week to acclimatize in animal housing conditions before being used for the experiment. All rats were fed with a regular diet and drinking water ad libitum during the adapting period.

2.6 Experimental design:

After the adaption period, rats were divided into regular diet group (negative control, *n =12)* and high-fat diet (HFD) group (*n = 72*). Rats in negative control were given regular diet, and the HFD rats were fed with high-fat diet for four weeks prepared by using saturated animal fat (*beef tallow*) to form 40% kcal of total daily fat [14]. Diabetes was induced four weeks later of HFD feeding, all 72 rats in HFD group intraperitoneally injected after 12h of fasting with previously prepared streptozotocin (40mg/kg) Followed by overnight administration of

oral 5% glucose solution to prevent the hypoglycemic shock. Seventy-two hours after streptozotocin administration, the rat with glucose levels > 200 mg/dL measured by a glucometer **(Accu-Chek, Roche, Basel, Switzerland)** was considered diabetic rat and selected for this study[17].

After diabetes induction, all 84 rats 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 twelve rats each per group; **Group II –** Non-treated 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 –** DM rats received oral 500 IU/kg of Vitamin D3 once daily plus rivastigmine 1 mg/kg/day. **Group VII –** T3D rats received oral rivastigmine 1 mg/kg/day.

The treatment period lasted for sixteen weeks, and rats were kept feeding on their respective diets until the end of the study. To treat each rat with a correct dose over the entire period of this study, vitamin D3 and rivastigmine doses were adjusted every two weeks according to the body weight changes.

2.7 Assessment of cognitive function:

2.7.1 Morris water maze (MWM) apparatus:

In this study, MWM was constructed out of a circular pool (147 cm in diameter and 60 cm in height) that filled with room-temperature water up to 10 cm below the rim. The water was made opaque by the addition of non-fat powdered Milk. A square platform (25-cm^2) was used to escape out of the maze.

2.7.2 Morris water maze (MWM) procedure:

The MWM test lasted seven days. On the first day, all rats were given rest upon the platform for 2 minutes to allow orientation to extra-maze cues before performing the MWM. After orientation, each rat underwent three training trials with a visible platform at intervals of 30 s. In each experiment, the rat was gently placed into the water facing the pool wall at one of three points of entry, Northeast (NE), Southwest (SW) and Southeast (SE). The escape latency was recorded for each trial. If the rat located the platform before 60 seconds had passed, if, after 60 seconds of swimming, the rat was gently guided to the platform and allowed to re-orient to the distal visual cues for 30 seconds, and the escape latency in these cases was recorded as 60 seconds then platform was submerged 1 cm below the water surface and water maze task was performed daily for the following four days. On the sixth day, a probe trial was conducted with no escape platform; each rat was placed into the pool from the start location at the quadrant opposite to the former platform quadrant which is southeast quadrant in our study[18,19]. Escape Latency, total time (seconds) whose rat spent in the northwest quadrant (target quadrant) and the number of crossings over the former platform location were calculated to assess cognition.

2.8 Hippocampus preparation and biochemical analyses:

Immediately after the MWM test, all rats were decapitation and hippocampi were immediately dissected, placed on ice, washed in cold 0.9% normal saline and weighed. Rats hippocampi subsequently homogenized in sodium phosphate buffer (pH 6.9) with a glass homogenizer on the ice and the homogenate centrifuged at 5,000 g. The clear supernatants obtained were used for estimate the hippocampal levels of; Aβ, iNOS, NO, TNF-α, IL-6, and

IL-1β were determined by quantification ELIZA kits following the company's recommended protocol[20].

2.9 Statistical analysis:

Statistical analysis was performed using SPSS (Statistical package of social sciences version 23). One-way analysis of variance (ANOVA) followed by Tukey HSD post hoc test for multiple comparisons. $P \leq 0.05$ was considered significant.

3. RESULTS

3.1 Effect of Vitamin D3 on cognitive dysfunction:

The development of cognitive dysfunction in rats was confirmed by a significant increase ($p<0.05$) of escape latency in non-treated diabetic rats for all training days compared to the negative control group. Diabetic rats treated with Vitamin D_3 plus rivastigmine exhibited a shorter time to find the hidden platform in training trials of MWM test throughout the 1^{st} and 2^{nd} training days compared with the positive control group ($p<0.05$). On the 3^{rd} training day, the time to escape from MWM was significantly reduced ($p<0.05$) in all vitaminD3 treated diabetic rats except vitamineD3 dose (100IU/kg/day) compared with a non-treated diabetic group ($p<0.05$). Moreover, the escape latency during 4^{th} and 5^{th} training days of MWM was significantly decreased in all vitamin D_3 treated diabetic rats compared with a non-treated diabetic group ($p < 0 .05$) (Fig.1).

Table 1 revealed that the number of times that rats crossed former platform location and the time spent in the target quadrant was significantly increased ($p < 0.05$) in all vitamin D3 treated diabetic rats except vitamin D3 (100 IU /kg /day) compared with a non-treated diabetic group. Moreover, time spent in the target quadrant was significantly increased in vitamin D_3 treated diabetic group (1000 IU /kg /day) ($p<0.05$), Vitamin D_3 plus rivastigmine treated diabetic group ($p<0.05$) compared to rivastigmine treated diabetic rats.

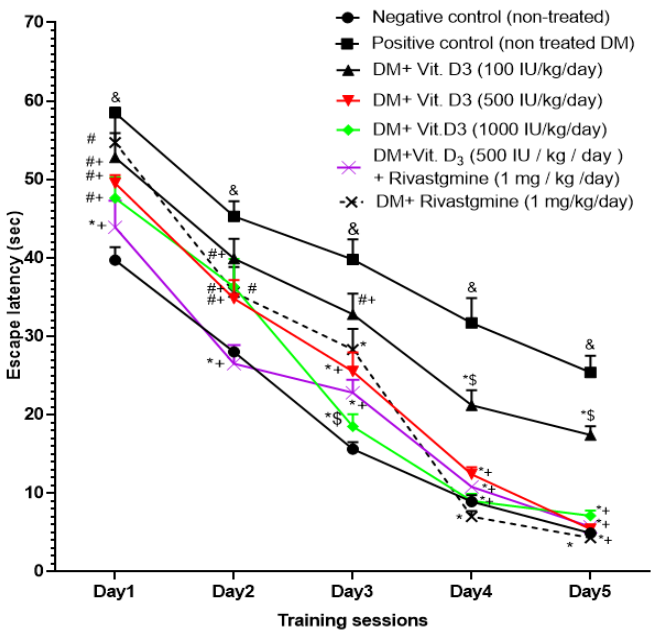

Fig. 1: Effect of different doses of Vitamin D3 (100, 500, 1000 IU/kg/day) on the escape latency of diabetic-induced Alzheimer in rats by MWM test .

*[&] P <0.05, compared with negative control group ; [#] P > 0.05, * P < 0.05 compared with positive control group; [+] P > 0.05, [$] P < 0.05 compared with rivastgminne group; ; by Tow-Way ANOVA and Tukey HSD post hoc test. Data expressed as the mean ± SEM; n = 10 rats.*

Table 1. Effect of different doses of Vitamin D3 (100, 500, 1000 IU/kg/day) on the number of times of crossing former platform location and time spent in the former platform quadrant of diabetic-induced Alzheimer in rats.

Groups		The number of times of crossing the former platform within 60 seconds.	Time spent in the former platform quadrant (sec)
Negative Control (non-treated)		9.33± 0.88	37.80 ±3.06
Diabetic groups	Positive control (non-treated)	3.83 ±0.31	16.67± 1.32
	Vit. D_3 (100IU/kg/day)	6 ±0.58 $^{\#+}$	22.47 ±1.10 $^{\#+}$
	Vit. D_3 (500 IU / kg / day)	7.83± 0.60 $^{*+}$	26.43 ±2.31 $^{*+}$
	Vit. D_3 (1000 IU/kg/day)	7.33 ± 0.62 $^{*+}$	33.72 ±2.01 $^{*\$}$
	Vit. D_3 500 IU / kg / day) + Rivastgmine (1 mg / kg /day)	7.33 ± 0.42 $^{*+}$	35.73 ±2.18 $^{*\$}$
	Rivastgmine (1mg/kg/day)	7.67± 0.56*	23.35 ± 1.63 $^{\#}$

Values are expressed as the mean ± SEM; n = 10 rats.

$^{\#}$ P > 0.05,*P < 0.05, compared with positive control group; $^{+}$ P > 0.05, $^{\$}$P <0.01 compared with rivastgminne group; by one -Way ANOVA and Tukey HSD post hoc test.

3.2 Effect of Vitamin D3 on Aβ overexpression:

β-amyloid peptide (Aβ42) exhibited twofold higher in non-treated diabetic rats compared with negative control group (14.60 pg\ml ±0.58; $p<0.05$ *versus* 6.20 pg\ml ± 0.42; table 2). This overexpression was significantly decreased ($P < 0.05$) in a dose-dependent manner by chronic administration of vitamin D3 doses (100, 500 and 1000 IU /kg /day) and Vitamin D_3 plus rivastigmine versus positive control. While a non-significant reduction of Aβ-42 level was obtained by administration of rivastigmine alone ($p>0.05$).

Table 2. Effect of different doses of Vitamin D3 (100, 500, 1000 IU/kg/day) on Aβ-42 level in hippocampal tissue of diabetic-induced Alzheimer in rats.

Groups		Aβ-42 (pg\ml)
Negative Control (non-treated)		6.20± 0.42
Diabetic groups	Positive control (non-treated)	14.60 ±0.58 $^{\&}$
	Vit. D_3 (100IU/kg/day)	11.76±0.43 $^{*\$}$
	Vit. D_3 (500 IU / kg / day)	11.78±0.47 $^{*\$}$
	Vit. D_3 (1000 IU/kg/day)	10.47±0.34 $^{*\$}$
	Vit. D_3 500 IU / kg / day) + Rivastgmine (1 mg / kg /day)	11.08±0.33 $^{*\$}$
	Rivastigmine (1mg/kg/day)	14.55±0.65$^{\#}$

Values are expressed as the mean ± SEM; n = 10 rats.

$^{\&}$ P <0.05, compared with negative control group ; $^{\#}$ P > 0.05, *P < 0.05, compared with positive control group; $^{\$}$P <0.01 compared with rivastgminne group; by one -Way ANOVA and Tukey HSD post hoc test.

3.3 Effect of Vitamin D3 on iNOS overexpression and NO overproduction :

Table 3 illustrated that the iNOS activity a 2.4 fold higher in non-treated diabetic rats (17.88 u\l ± 0.85 ; p<0.05) compared with a non-diabetic group (7.60 u\l ± 0.79) and this upregulation was significantly alleviated (p<0.05) in all vitamin D3 treated diabetic rats compared to positive control. Also, In compared with rivastigmine group, an only higher dose of vitamin D3 group (1000 mg/kg/day) was exhibited a significant reduction of inducible nitric oxide synthase level (p < 0.05). Moreover, The results from Table 2 showed that all diabetic rats treated with vitaminD3 produced a significant decrease (p<0.05) of nitric oxide level versus a positive control group.

Table 3. Effect of different doses of Vitamin D3 (100, 500, 1000 IU/kg/day) on iNOS and NO level in hippocampal tissue of diabetic-induced Alzheimer in rats.

Groups	iNOS (u\l)	NO (ppb)
Negative Control (non-treated)	7.60 ± 0.79	20.54± 0.53

Diabetic groups	Positive control (non-treated)	17.88± 0.85[&]	27.09 ± 0.57[&]
	Vit. D_3 (100IU/kg/day)	9.17 ± 0.87 *+	21.19 ±0.52*+
	Vit. D_3 (500 IU / kg / day)	9.86 ± 0.47*+	21.50 ± 0.43 *$
	Vit. D_3 (1000 IU/kg/day)	7.88 ± 0.61*$	22.75 ±0.52 *+
	Vit. D_3 500 IU / kg / day) + Rivastgmine (1 mg / kg /day)	11.38± 0.68*+	20.96± 0.71 *+
	Rivastgmine (1mg/kg/day)	11.36± 0.45 *	22.08 ±1.01 *

Values are expressed as the mean ± SEM; n = 10 rats.

[&] P <0.05, compared with negative control group ,[*] P < 0.05, compared with positive control group ;[+] P > 0.05, [$] P <0.01 compared with rivastgminne group; by one -Way ANOVA and Tukey HSD post hoc test.

3.4 Effect of Vitamin D3 on Proinflammatory cytokines :

Table 4 showed that the DM was correlated with a significant expression increase ($p < 0.05$) of pro-inflammatory cytokines; TNF- α, IL-6 and IL-1β (15.97 pg\ml ± 0.42, 10.77 pg\ml ±0.47 and 155.70 pg\ml ± 5.42, resp.) in hippocampal tissues of non-treated diabetic rats compared with negative control group (5.95 ±0.33, 4.90±0.29 and 119.18±1.64, resp.). Concomitantly, the mean hippocampal level of NF- α, IL-6 and IL-1β in diabetic rats chronically treated with vitamin D3 were significantly reduced ($p<0.05$) compared to the non-treated diabetic group**.** We also observed that diabetic rats treated with different doses of vitamin D3 (500 and 1000 IU /kg /day) exhibited a significantly lower level of TNF- α ($p<0.05$) compared to rivastigmine group.

Table 4. Effect of different doses of Vitamin D3 (100, 500, 1000 IU/kg/day) on TNF- α, IL-6, and IL 1β level in hippocampal tissue of diabetic-induced Alzheimer in rats.

Groups		TNF- α (pg\ml)	IL-6 (pg\ml)	IL 1β (pg\ml)
Negative Control (non-treated)		5.95 ±0.33	4.90±0.29	119.18±1.64
Diabetic groups	Positive control (non-treated)	15.97±0.42[&]	10.77±0.47[&]	155.70±5.42[&]
	Vit. D_3 (100IU/kg/day)	10.86±0.30 *+	6.02±0.42 *+	121.44±2.72 *+
	Vit. D_3 (500 IU / kg / day)	7.88 ±0.32 *$	6.90±0.49 *+	125.50±3.43 *+
	Vit. D_3 (1000 IU/kg/day)	7.87 ±0.50 *$	4.99±0.48 *+	120.70±2.12 *+
	Vit. D_3 500 IU / kg / day) + Rivastgmine (1 mg / kg /day)	9.98 ±0.42 *+	6.41±0.50 *+	125.25±3.20*+
	Rivastgmine (1mg/kg/day)	11.21 ±0.54 *	6.73±0.63 *	122.40±1.81 *

Values are expressed as the mean ± SEM; n = 10 rats.

[&] P <0.05, compared with negative control group ; [*] P < 0.05, compared with positive control group ;[+] P > 0.05, [$] P <0.01 compared with rivastgminne group; by one -Way ANOVA and Tukey HSD post hoc test.

4. DISCUSSION:

The obtained data from the present study clearly demonstrated that (1) HFD and STZ injection overexpressed Aβ, iNOS, NO, TNF-α, IL-6, and IL 1β an in the hippocampus (2) Chronic treatment with vitamin D3 significantly and dose-dependently alleviated cognitive deficits, with significant decreases in Aβ42 as well as suppressing nitric oxide synthase pathway via attenuated hippocampal iNOS and NO overproduction and mitigation of inflammation state of diabetic rats brains by significantly decreased the levels of pro-inflammatory markers TNF- α , IL-6 and IL 1β as underlying mechanism for the resulted improvement .

MWM test is the most reliable behavioral test in the experimental animal field. MWM monitoring spatial learning and memory capacities and is vastly thought to have a connection for hippocampal-dependent memory in human being [21]. Our study illustrated that vitaminD3 treatment significantly prevented learning and memory deficits in diabetic rats by decreasing escape latency and increasing the time spent in the target quadrant and the number of times crossing the platform in all vitamin D3 groups versus positive control. This finding illustrates the crucial role of vitamin D3 in the enhancement of hippocampus-dependent learning and memory. A previous investigation demonstrated that vitamin D deficiency rats had significantly lowered escape performance with higher navigation failure in MWM versus healthy rats which is consistent with the results described here[22]. In line, Latimer et al., reported the superiority of a high vitamin D diet over low vitamin D diet (1,000 and 100 IU / Kg, respectively) in markedly enhancing of MWM escape performance [23].

Inducible nitric oxide synthase (iNOS) catalyzes the synthesis of nitric oxide (NO). NO has been proven to be a crucial signaling molecule involved in synaptic plasticity and memory [24]. NO, consider the main Aβ -induced component that contributes to the immune response. iNOS and Aβ were reported to induce the accumulation of each other. During diabetes mellitus, reactive oxygen species and nitrogen species (RONS) generated by damaged microglia, neurons, and astrocytes may elevate the expression of iNOS. As a consequence, higher NO level leads to cell death via prevention of mitochondrial and neuronal respiration, further resulting in Aβ accumulation and neuronal toxicity [25,26].

In the current study, the contribution of nitric oxide signaling on Aβ accumulation and pathogenesis AD was evidenced by significantly increased ($P<0.05$) of iNOS activity, NO production and Aβ deposition of hippocampal tissue in non-treated diabetic rats compared with non-diabetic rats. These results go hand in hand with previous reports that also showed an elevated level of Nitric oxide synthase activity in leukocytes and brain microvessels of patients with AD [27] . Similarly, Dursun *et al.*, demonstrated that Aβ significantly induced iNOS mRNA and protein expression in AD patients [28].

To our knowledge, no previous study has assessed the direct effect of vitaminD3 on brain level of iNOS and NO of diabetic rats. In the present study, Chronic administration of vitamin D3 significantly decreased iNOS ($p<0.05$), NO($p<0.05$) and Aβ($p<0.05$) levels compared to the positive control group. In accordance with our work, Dursun E et al., reported that that 1,25 (OH) 2D3 prevents Aβ-induced iNOS expression and that iNOS expression is regulated by the vitamin D-VDR pathway in cortical neurons. Additionally, disruption of this

pathway resulted in the induction of iNOS expression regardless of the presence of Aβ [29]

In addition to being a progressive neurodegenerative disorder, AD is considered to be an inflammatory brain disease due to the recruitment of reactive astrocytes and microglia around β amyloid plaques, the major pathological hallmark of AD[30,31]. Elevated levels of cytokines and chemokines around β amyloid plaques in AD also contribute to the immune response in AD brains[32,33]. Increased levels of proinflammatory cytokines such as TNF-*α*, interleukin-6 (IL-6), and interleukin-1*β* (IL-1*β*) have been observed in DM and play a critical role on the pathogenesis of DM and AD [34]. TNF-α magnifies inflammatory pathway via multiple mechanisms, such as microglial cells stimulation that kill neighboring neurons by reactive oxygen species (ROS) releasing, proteolytic enzymes liberation, elevation of βAPP production, and accelerates the buildup of insoluble Aβ peptide from βAPP [35]. This insoluble peptide (Aβ) directly binds to the microglia cell surface receptors and activates nuclear factor κB (NF-κB), further elevating cytokines production resulting in a downward spiral of chronic inflammation. Also, astrocyte is another cell type involved in the pathogenesis of AD [36]. Proinflammatory cytokines such as IL-1β and IL-6 activated astrocytes then the activated astrocytes increase inflammation via the releasing of cytokines such as tumor necrosis factor α (TNF-α) and IL-6 [37].

The TNF-α pathway modification led to the amelioration in the cognitive capabilities of experimental models. Furthermore, the neuropathological parameters of AD such as aggregation of phosphorylated tau protein, Aβ deposition, and activated microglial and astrocyte cell were all observed to be reduced by the inhibition of the TNF-α pathway. It's worth to mention that prevention of this signaling cascade inhibits the strong stimulation of microglial cells, keeping them in a state of moderate stimulation where they play a neuroprotective role by elevating of βAPP clearance [38].

In the present work, besides the cognitive dysfunction produced by DM, hippocampal levels of pro-inflammatory cytokines TNF- α, IL-6, and IL-1β were significantly increased ($p<0.05$) in non-treated diabetic rats compared with negative control group. Consistent with our work, studies found that chronic hyperglycemia promotes the generation of different intracellular inflammatory pathways, including inflammatory mediators such as TNF-*α*, IL-6, and C-reactive protein (CRP) [39,40]. Similarly, a study by S. Takeda et al. in a transgenic mouse model of AD and diabetes reported that Aβ- induced synaptic dysfunction was positively correlated with elevated levels of cytokines and tumor necrosis factor (TNF- *α*) [41].Also, Shamim *et al*.,. reported that high levels of proinflammatory cytokines; TNF-α, IL-1β, and IL-6 might prevent amyloid Aβ phagocytosis in brains of astrogliosis patient and led to neural death. [42]. Moreover, Birch et al., (2014) proven a direct correlation between proinflammatory cytokines and Aβ production by observing that TNF-α and IFN- γ transcriptionally overproduce β-secretase, beta site amyloid precursor protein cleaving enzyme 1 (BACE1) [43].

Our study indicate that vitaminD3 showed an anti-inflammatory effect as proven by the significantly decreased ($p<0.05$) hippocampal level of NF- α , IL-6 and IL-1β in diabetic rats chronically treated with different doses of vitamin D3 (100, 500 and 1000 IU /kg /day) , vitamin D3 plus rivastigmine group and rivastigmine group compared to non-diabetic rats. These findings are in agreement with those of Erbaş *et al.*, (2014) who found that vitamin D3 ameliorate cognitive dysfunction and decreases inflammation of the fatty liver in a rat model of metabolic syndrome [44]. In line, Tse *et al.*, (2018) reported that the Administration of 1,25(OH)D for 3 weeks decreased pro-inflammatory cytokine Il-1β amyloid burden of aged rats (20 months). Also, multiple studies reported the useful role of vitamin D on the prevention of inflammation[45]. Clinical studies demonstrate decreased expression of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6 in higher vitamin D level

individuals compared to those who experienced vitamine D deficiency. Based on some other animal studies, vitamin D, through selective blockage of the NF-kB signaling pathway, results in a significant reduction in inflammatory IL-1β and TNF-α expression[46].

The reduction in TNF- α, IL-6 and IL-1β of vitaminD3 treated diabetic rats in the current study, supporting vitaminD3 antiinflammatory actions that could be of therapeutic relevance in preventing the progression of Alzheimer.

4. CONCLUSION

The results of this research suggest that upregulation of nitric oxide synthase pathway along with the increase in pro-inflammatory cytokines is critically involved in cognitive dysfunction associated with diabetes. VitaminD3 can ameliorate these effects and has a promising neuroprotective effect in diabetic-induced cognitive dysfunction.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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