



## **Study the Role of Vitamin D on Some Brain Degenerative Disorders in Male Albino Rats**

**Fatma Fayed, Sahar Elsayy, Mohamed Shebl, Haidy Khattab**

*Medical Physiology Department, Faculty of Medicine, Tanta University*

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### **Abstract**

**Background:** Vitamin D, a steroid hormone that plays an important role in bone and calcium metabolism, now is known it has different beneficial functions and actions on various tissues and cell types. There are evidences that vitamin D implies some functions in the central nervous system as a neurosteroid hormone. **Aim:** Study the role of vitamin D on aluminum chloride (AlCl<sub>3</sub>) induced brain degeneration in male albino rats. **Materials and Methods:** This study was carried out on 50 adult male Wistar albino rats. The rats were divided into three groups: Group I (Control group) received normal saline followed by corn oil. Group II (AlCl<sub>3</sub> treated group) received AlCl<sub>3</sub> followed by corn oil. Group III (VD<sub>3</sub> treated group): subdivided into Group IIIa received VD<sub>3</sub> followed by both AlCl<sub>3</sub> and VD<sub>3</sub>. Group IIIb: received AlCl<sub>3</sub> followed by VD<sub>3</sub>. Behavioral tests were done. Brain tissue acetylcholinesterase activity, malondialdehyde and glutathione peroxidase were assessed. **Results:** The results revealed that in AlCl<sub>3</sub> treated group, there was a significant decrease in acetylcholinesterase level and glutathione peroxidase and a significant increase in malondialdehyde compared to the control group and significant increase in acetylcholinesterase level and glutathione peroxidase, and a significant decrease in malondialdehyde in VD<sub>3</sub> treated group compared to AlCl<sub>3</sub> treated group; there was also improvement in behavioral parameters in VD<sub>3</sub> treated group compared to AlCl<sub>3</sub> treated group. **Conclusion:** We concluded that either the protective or the therapeutic effect of vitamin D produced significant improvement in motor impairment, learning, and memory.

## INTRODUCTION

Vitamin D, a steroid hormone that plays an important role in bone and calcium metabolism, it has different beneficial functions and actions on various tissues and cell types.(1)

Researches suggest that vitamin D plays an essential role in preventing and/or decreasing the risk of a variety of neurodegenerative disorders. The risk of neurodegenerative pathologies may be increased by Vitamin D deficiency. (2)

1 $\alpha$ , 25-dihydroxy-vitamin D or simply calcitriol, is the active form of vitamin D (VD3) plays a pivotal role to maintain the normal neuronal function, such as immunomodulation and detoxification processes.(3)

Anti-oxidants activity is one of the important effects of VD3; it improves brain enzyme activity by reducing lipid peroxidation. VD3 deficiency will result in dysregulation in reactive oxygen species (ROS) and Ca<sup>2+</sup> signaling pathways. In some neurodegenerative pathologies as Alzheimer's disease, it might initiate beta-amyloid formation, which progresses to neuronal death and dementia .(4)

The proposed mechanism of action of VD3 is explained by its ability to bind to the activated VD3 receptor/retinoic X receptor heterodimeric complex to specific DNA sequences that control gene expression.(5) VD3 probably affects genes involved in glutamatergic and GABA-ergic neurotransmissions, calcium regulation, as well as neurotrophic factors and genes involved in neuroprotection. (6)

VD3 is necessary for brain function, like, neurotransmission, synapse formation, synaptic plasticity, and dendritic arborization and its

deficiency may have a negative effect on these critical processes. (7) Furthermore, VD3 deficiency acts as a risk factor for Parkinson's disease development. (8)

There are many environmental toxicants that have the capacity to impair the brain. Aluminum (Al) is the most abundant metal present on the earth's crust. It is extensively used in daily life and was found in drinking water probably due to water purification procedures, the new 20th century industrial products containing Al salts. Aluminum's slow progressive uptake into the brain over a long prodromal phase. (9)

We designed the current study to assess the effect of vit D on aluminum chloride induced brain damage in male albino rats.

There is controversy about the relation of vitamin D status and cognitive function and whether its deficiency is the cause of the disease progression. (10)

### Material and Methods:

#### Chemicals and Reagents:

##### Vitamin D:

Vitamin D (Deverol S®, colecalciferol, VD3) was purchased from Mephis company as 2 ml ampoule containing 5 mg equivalent to 200,000 IU (1 IU is the biological equivalent of 0.025  $\mu$ g), each ampoule was diluted by corn oil from Sigma – Aldrich to give a concentration of 0.125 $\mu$ g/ml. (11)

##### Aluminum chloride:

AlCl<sub>3</sub> (1 AlCl<sub>3</sub>: 6 H<sub>2</sub>O) was purchased from Sigma –Aldrich as powder 1Kg is prepared by dissolving 2.414gm of salt per litre of distilled water.

##### Experimental Animals:

This study was carried out on 50 adult male Wistar albino rats (weighing 150 - 200g). All rats were

housed in the animal house at the Faculty of Medicine Tanta University in cages measured 70×70×60 cm 5 animals per cage under a standard animal laboratory room temperature with free access to water and food. All procedures were subjected to the ethical committee of Tanta University approval number 32902/2/19. The duration of the research started from February 2019 till February 2020.

#### Experimental design:

The rats were divided into three groups as the following:

**Group I (Control group)** (10 rats): They received intraperitoneal (IP) injection of 1ml normal saline once daily for two weeks followed by intramuscular (IM) injection of 0.5 ml/kg corn oil once daily for two weeks. <sup>(12)</sup>

**Group II (AlCl<sub>3</sub> treated group)** (20 rats): These animals received IP injection of (AlCl<sub>3</sub>) at a dose of 7 mg/kg body weight once daily for two consecutive weeks followed by IM injection of 0.5 ml/kg corn oil for two weeks. <sup>(13)</sup>

**Group III (VD3 treated group)** (20 rats): The animals of this group were subdivided into:

**Group III a** (10 rats): These animals were received IM injection of VD3 at a dose of 1µg/kg once daily for 7 days, followed by injection of both AlCl<sub>3</sub> at a dose of 7 mg/kg body weight once daily and the same dose of VD3 for two weeks then rats have received IP injection of 1ml normal saline once daily for one week. <sup>(3)</sup>

**Group III b** (10 rats): The animals of this group were received IP injection of AlCl<sub>3</sub> at a dose of 7 mg/kg body weight once daily for two consecutive weeks followed by IM injection of VD3 at a dose of 1µg/kg once daily for two weeks. <sup>(4)</sup>

At the end of the experimental period ten rats died from aluminum chloride treated group ,the final numbers were, group I=10, group II=10, group IIIa=10 and group IIIb=10

We gave each substance throughout the day according to **table (1)**.

All the animals from all groups were subjected for:

**Table (1) Time table of given each substance through out the day**

Substance injected	Group I	Group II	Group IIIa	Group IIIb
Normal saline in the first two weeks	9 A.M			
Corn oil in the last two weeks	9 A.M			
AlCl <sub>3</sub> in the first two weeks		9 A.M		
Corn oil in the last two weeks		9 A.M		
VD3 for 7 days			9 A.M	
AlCl <sub>3</sub> in 2 <sup>nd</sup> & 3 <sup>rd</sup> weeks & VD3 in 2 <sup>nd</sup> & 3 <sup>rd</sup> weeks			8 A.M 9 A.M	
normal saline for the 4 <sup>th</sup> week			9 A.M	
AlCl <sub>3</sub> for 1st two weeks				8 A.M
VD3 for last two weeks				9 A.M

#### Behavioral tests:

**1.Locomotor Activity Test (Open field test):** This test was performed in order to evaluate the exploratory behavior, as an index of

spontaneous motor activity and was done according to protocol described by Broadhurst,(14).

Locomotor activity of the rat was measured using the open field test. During this test, animals were allowed to freely explore the apparatus for 15 min. Each rat was placed in the center of the open field and the distance traveled every 5 min was recorded for 15 min. After the behavioral evaluation of each rat, the apparatus was cleaned with 5% ethanol solution.

**2. Forced swimming test :** This test was centered on animal's response to the threat of drowning, whose result had been interpreted as measuring susceptibility to negative mood and was done according to procedure described by *Castagné et al.*, (15).

The animals were placed individually into a cylinder (40 cm height and 23 cm diameter), containing water up to 25 cm below the top. The immobility time was monitored for 5 (min), after an initial 1 min adaptation period. The reduction of this immobility time is suggestive of an antidepressive-like action.

**3. T-maze test:** Elevated T-maze (ETM) In this study, the ETM was used to assess learning and memory features in rats. The experimental protocol was based on the report of *Conde et al.*, (16).

In the training session, which was performed only on one day, rats were re-exposed to the ETM as many times as needed to remain in the enclosed arm for 300 s (avoidance criterion). The number of trials to reach the avoidance criterion was used to assess learning. The time in which the animal remained in the enclosed arm was recorded for each trial (avoidance latency).

Twenty-four hours after training (test session), rats were re-exposed to the enclosed arm during two subsequent trials (i.e., test and retest). The time that the animal remained in the enclosed arm was

recorded and used to assess memory retrieval. Experiments were performed in a dim light and quiet room with an observer inside the room (3 m×3 m) seated at least 2 m from the open arms of the apparatus.

All experiments were performed between 13:00 and 17:00. The apparatus was cleaned with 5% ethanol solution between subjects.

#### **Tissue sample preparation:**

After anesthetization by IP injection of pentobarbital at a dose of 800 mg/kg (17), animals were sacrificed. The brains were immediately removed, placed in ice-cold isotonic saline and the cerebrum was dissected then stored at  $-80^{\circ}\text{C}$ . Later the brain tissue was taken and minced into small pieces, which were later used for the estimation of the following parameters:

- **Acetylcholinesterase (AChE)** activity according to the method described by **Magnotti et al.**, (18).
- **Glutathione Peroxidase (GPX)** according to the method described by **Rotruck et al.**, (19).
- **Malondialdehyde (MDA)**, according to the method described by **Ohkawa et al.**, (20).

The sacrificed animals were packed in a special package according to safety precautions and infection control measures and were sent with hospital biohazard.

#### **Statistical analysis:**

The data were analyzed using Statistical Program for Social Science (SPSS) version 22.0. Quantitative data were expressed as mean  $\pm$  standard deviation (SD).

ANOVA and Tukey tests were used to compare the groups. P value less than 0.05 indicates significance.

### **Results:**

#### **I-Animal survival**

Ten rats died from aluminum chloride treated group.

#### **II-Behavioral tests:**

##### **1-Open field test (distance/time) (mm/15 minute) ( table 2):**

Locomotion (distance travelled every 5 min for 15 min) in animals of group II showed a significant decrease ( $P \leq 0.05$ ) when compared to that of group I; however, it showed a significant increase ( $P \leq 0.05$ ) in group IIIa and group IIIb when compared to that of group II moreover, they showed a significant increase ( $P \leq 0.05$ ) in group IIIa when compared to that of group IIIb.

##### **2-Forced Swimming test (immobility time) (seconds/5minute) ( table 2):**

Immobility time in animals of group II showed a significant increase in ( $P \leq 0.05$ ) when compared to that of group I; however, it showed a significant decrease ( $P \leq 0.05$ ) in group IIIa and group IIIb when compared to that of group II, there also significant decrease ( $P \leq 0.05$ ) in group IIIa when compared to that of group IIIb.

##### **3-T-maze test: Elevated T-maze (ETM) task**

###### **A-Training session: number of trials ( table 2):**

The number of trials in animals of group II showed a significant increase ( $P \leq 0.05$ ) when compared to that of group I; however, it showed a significant decrease ( $P \leq 0.05$ ) in group IIIa and group IIIb when compared to that of group II; moreover, they

showed a significant decrease ( $P \leq 0.05$ ) in group IIIa when compared to that of group IIIb.

###### **B-Retest Session ( table 2):**

The time animals remain in enclosed arm in retest session in animals of group II showed a significant decrease ( $P \leq 0.05$ ) when compared to that of group I; however, it showed a significant increase ( $P \leq 0.05$ ) in group IIIa and group IIIb when compared to that of group II; moreover, they showed a significant increase ( $P \leq 0.05$ ) in group IIIa when compared to that of group IIIb.

#### **III-Tissue AChE , glutathione peroxidase and Malondialdehyde activity:**

##### **1- Tissue AChE activity and tissue glutathione peroxidase activity in all studied groups( table (3):**

It showed a significant decrease ( $P \leq 0.05$ ) in tissue Ach E activity and tissue glutathione peroxidase activity in animals of group II when compared to that of group I; however, it showed a significant increase ( $P \leq 0.05$ ) in group IIIa and group IIIb when compared to that of group II; moreover, they showed a significant increase ( $P \leq 0.05$ ) in group IIIa when compared to that of group IIIb.

##### **2-Tissue Malondialdehyde (MDA) activity in all studied groups Table (3):**

It showed a significant increase ( $P \leq 0.05$ ) in tissue MDA activity in animals of group II when compared to that of group I; however, it showed a significant decrease ( $P \leq 0.05$ ) in group IIIa and group IIIb when compared to that of group II; moreover, they showed a significant decrease ( $P \leq 0.05$ ) in group IIIa when compared to that of group IIIb.

**Table (2): Open field test (distance/time) & forced Swimming test (immobility time) and T-maze test in all studied groups :**

Groups	Group I(10rats) (control) mean $\pm$ SD	Group II(10rats) (AlCl <sub>3</sub> ttt) mean $\pm$ SD	Group III (Vit D ttt)	
			IIIa(10rats) mean $\pm$ SD	IIIb (10rats) mean $\pm$ SD
Open field test (distance/time) (mm/15 minute)	58.6 $\pm$ 2.22	43 $\pm$ 2.00*	57.5 $\pm$ 2.41 <sup>#Δ</sup>	52.4 $\pm$ 2.06 <sup>#</sup>
Forced Swimming test (immobility time) (seconds/5minute)	44.7 $\pm$ 3.05	85.5 $\pm$ 3.68*	46.7 $\pm$ 2.58 <sup>#Δ</sup>	56.4 $\pm$ 2.67 <sup>#</sup>
T-maze test: numbers of trials	7.2 $\pm$ 1.619	14.3 $\pm$ 1.160*	8.2 $\pm$ 1.169 <sup>#Δ</sup>	11.5 $\pm$ 0.527 <sup>#</sup>
T-maze: Re test Session ( seconds)	175.5 $\pm$ 3.02	145.7 $\pm$ 3.59*	173.5 $\pm$ 2.72 <sup>#Δ</sup>	164.5 $\pm$ 3.03 <sup>#</sup>

\* #Δ denotes statistical significance (P $\leq$  0.05)

\* versus Group I

# versus Group II

Δ Group IIIa vs Group IIIb

ttt treated

**Table (3): Tissue AChE activity & Glutathione Peroxidase (GPX) and Malondialdehyde in all studied groups :**

Parameters	Group I (10 rats) (control) mean $\pm$ SD	Group II (10 rats) (AlCl <sub>3</sub> ttt) mean $\pm$ SD	Group III (Vit D ttt)	
			IIIa (10 rats) mean $\pm$ SD	IIIb (10 rats) mean $\pm$ SD
Tissue Ach E activity (mU/ml/g)	896.32 $\pm$ 0.18	893.55 $\pm$ 0.37*	896.28 $\pm$ 0.18 <sup>#Δ</sup>	895.74 $\pm$ 0.32 <sup>#</sup>
Glutathione Peroxidase (GPX) nmol/min /mg of protein	1.32 $\pm$ 0.17	0.52 $\pm$ 0.16*	1.29 $\pm$ 0.16 <sup>#Δ</sup>	0.87 $\pm$ 0.05 <sup>#</sup>
Malondialdehyde (MDA) (nmol/g)	6.38 $\pm$ 0.19	7.49 $\pm$ 0.34*	6.42 $\pm$ 0.18 <sup>#Δ</sup>	6.72 $\pm$ 0.17 <sup>#</sup>

\*#Δ denotes statistical significance (P $\leq$  0.05)

\*versus Group I

# versus Group II

Δ Group IIIa vs Group IIIb

ttt treated

### Discussion:

Vitamin D hormone is essential for growth and differentiation in a variety of organs, including the brain. Numerous data suggest that vitamin D plays an important role in the brain through the induction of many CNS genes, neurotrophins release and activity of key neurotransmitter metabolism enzymes (21). Aluminum chloride is a well-known neurotoxicant reported to accelerate oxidative damage to biomolecules. (22)

Our aim is to study the prophylactic and therapeutic role of VD3 in aluminum chloride induced brain degeneration.

Our study confirmed that injection of AlCl<sub>3</sub> in rats causes brain degeneration, evidenced by a decrease in tissue AChE level.

In agreement with our results, Taïr et al., (23) found that AlCl<sub>3</sub> administration in rats produced a significant decrease in cerebrum AChE activity compared to control rats.

The explanation of our results is that exposure of rats to AlCl<sub>3</sub> caused accumulation of the neurotoxicant at different levels in the brain, affecting even serotonergic neurotransmission.(20)

In disagreement with our results, Al exposure leads to an increase in AChE activity in the brain of rats. (21) This could be explained by the hypothesis of the biphasic effect of aluminum related to the metal exposure duration established by Gabriela et al. (24)

Treatment by Vit D showed a significant increase in tissue AChE level; our study also showed that Vit D has a protective effect against aluminum chloride toxicity as evidenced by a significant increase in AChE level in group IIIa compared with IIIb.

In agreement with our results, Medhat et al., (25) found that AChE showed a significant increase in VD3 treated group compared to Alzheimer group.

Zhang and Greenberg, (26) reported that the human AChE promoter contains many binding sites for osteogenic factors, including 1,25-(OH)<sub>2</sub>VD<sub>3</sub> and 17 $\beta$ -estradiol.

Our results regard behavioral tests showed a significant increase (immobility time in forced swimming test and a number of trials and a significant decrease in (locomotion in open field test and time animals remain in enclosed arm in test and retest session) in AlCl<sub>3</sub> treated group compared to control group. Aluminum chloride was found to decrease the crossing scores according to the results obtained after performing in the open field apparatus.

Similar observations have also been reported by Colomina, et al., (27), who noticed a significant

reduction in spontaneous locomotor activity after aluminum treatment of rats.

In disagreement with our results, Berihu et al., (28) showed that Al increased the activity scores of locomotor activity. The hyperactivity observed in these tests were considered to be the result of stress conditions.

An explanation of our findings may be that Al competes in the biological systems with cations, especially with magnesium. Also, aluminum binds to transferrin and to citrate in the plasma, and it can also influence the secondary transmission systems and availability of calcium (29).

Scientific data showed that Al interferes with the main neurotransmitters regarding their synthesis, storage, release, activation or inactivation of their receptors (30).

Observations and studies show that aluminum intoxication decreases by 40% the level of dopamine in the substantia nigra and causes an imbalance of dopamine metabolites, suggesting alteration of its metabolism. (31)

Cordeiro et al., (30) showed that Al disturbs the Ca<sup>2+</sup>/calmodulin signal-dependent of calcineurin that regulates the process of passing the system via the synaptic membrane.

In agreement with our results, Berihu, et al., (28) found that the result of the forced swimming test showed a significantly increased immobility time of aluminum-treated mice.

A study in which Al was administered intraperitoneally showed that the metal produces a decrease of density in dopamine D1 and D2 receptors from the cortex. (32)

Administration of AlCl<sub>3</sub> to rats led to decreased levels of serotonin and its metabolite 5-hydroxy-indoleacetic acid in the cortex; these changes are

related to the inhibitory effect of aluminum on serotonin by cholinergic input loss .(33)

However, Berihu et al., (28) concluded that AlCl<sub>3</sub> exposure has negative effects on the anxiety-related behavior of rats, as indicated by the increased rate of anxiety in Al treated rats.

Explanation of these results may be due to a decline in learning ability induced by the deterioration of hippocampal function.(34)

Our results regard behavioral tests showed a significant increase in (locomotion in open field test and time animals remain in enclosed arm in test and retest session) and a significant decrease in( immobility time in forced swimming test and the number of trials) in VD<sub>3</sub> treated group compared with group AlCl<sub>3</sub> treated group and significant change in group IIIb compared with group IIIa.

The dopamine metabolism in the forebrain is affected by VD<sub>3</sub> deficiency with a decreased conversion of 3, 4-dihydroxyphenylacetic acid (DOPAC) to homovanillic acid HVA. (35)

Eyles et al., (36) showed that the substantia nigra presents one of the highest brain concentrations of VDR, indicating the importance of this mediator in dopaminergic pathways.

The rate-limiting enzyme for dopamine synthesis Tyrosine hydroxylase (TH) might be directly modulated by VD<sub>3</sub>, as observed by immunohistochemistry staining. (37)

Cui et al., (38) demonstrated that VD<sub>3</sub> partially restores TH protein and TH-immunoreactive fibers. These effects also increased the glial-derived neurotrophic factor (GDNF) protein, contributing to VD<sub>3</sub> neuroprotective effects on dopaminergic neurons.

Orme et al., (39) demonstrated that VD<sub>3</sub> increase in the number of dopaminergic neurons and upregulation of GDNF protein.

Mahar et al., (40) suggested that VD<sub>3</sub> signaling could be regulated in the brain in response to stress, one of the major environmental factors associated with the occurrence of major depressive disorder episodes.

Latimer et al., (41) examined the effects of long-term dietary manipulation of serum VD<sub>3</sub> and tested the hypothesis that cognitive decline with ageing can be slowed or prevented by higher VD<sub>3</sub> levels; the results of the study provide evidence of a potential cause-and-effect relationship because raising VD<sub>3</sub> levels prevented age-related cognitive decline.

Medhat et al., (24) confirm the effective role of VD<sub>3</sub> in the improvement of the cognitive functions of brain treated rats.

The mechanisms by which the VD<sub>3</sub> undergoes the behavioral aspects of the neurocognition, neuro-protection are through anti-oxidative, immune-modulation, (42) neuronal calcium regulation, enhanced nerve conduction and detoxification.(43) VD<sub>3</sub> up-regulates gamma glutamyl transpeptidase (44), increasing anti-oxidant glutathione. Additionally, similar to the benefits of traditional anti-oxidant nutrients, VD<sub>3</sub> inhibits inducible NO syntheses (45) that serves to protect the brain from free radicals-induced damage. VD<sub>3</sub> has been also shown to attenuate the neurotoxicity of 6-hydroxydopamine exposure in rats (41). These neuroprotective effects influence the cognitive function of the brain.

VDR and catalytic enzymes are co-localized in the areas of the brain involved in complex planning, processing, and formation of new memories. (47)



VD3 up-regulates neurotrophin factors such as NT-3 (48). NT-3 is found in the hippocampus, which is especially sensitive to age or pathology-related degeneration (49) and neocortex and protects nerve transmission and synaptic plasticity. (43)

Our study revealed that AlCl<sub>3</sub> affects oxidative stress evidenced by increased tissue MDA level and decrease in tissue Glutathione Peroxidase activity in AlCl<sub>3</sub> treated group compared with the control group. In agreement with our results, similar results were mentioned by Nedzvetsky et al., (50). (who stated that Al-induced a significant increase in MDA concentration in the frontal cortex of rats.

Nearly similar findings were obtained by Manal et al., (51) they demonstrated that administration of AlCl<sub>3</sub> induced elevation of LPO in the brain of Al-treated rats. This is evidenced by the increased production of MDA, a negative correlation between Al exposure and GPX activities.

In addition, Yang et al., (52) reported that intraperitoneal injection of AlCl<sub>3</sub> could accelerate lipid peroxidation in rat's brain.

All of these records are in agreement with the fact that Al reduced the total anti-oxidant parameter levels, enhancing the imbalance between pro-oxidant and anti-oxidant potentials (53).

The possible mechanisms for the elevation of lipid peroxidation are the binding of Al to negatively charged brain phospholipids, which contain polyunsaturated fatty acids and are easily attacked by ROS such as O<sup>2</sup>·, H<sub>2</sub>O<sub>2</sub>, and OH· which leads to an increase in lipid peroxidation. Another mechanism may be the direct neurotoxic effect of Al or perhaps a disarrangement of the cell

membrane caused by increased lipid peroxidation. (54)

In our study tissue, MDA level showed significant decrease and tissue Glutathione Peroxidase activity showed a significant increase in VD3 treated group compared to AlCl<sub>3</sub> treated group but tissue MDA level still significant increase and tissue Glutathione Peroxidase activity still a significant decrease in group IIIb compared with group IIIa.

In agreement with our results, Lima et al., (55) found that VD3 treatments significantly decreased nitrite contents and lipoperoxidation as related to the non-treated 6-OHDA rats, indicating that VD3 presents an anti-oxidant action.

The VD3 treatment was shown to increase cell viability and decrease ROS production. (56)

Hajiluan et al., (57) found that VD3 improved hippocampus oxidative stress and inflammatory markers in high-fat diet HFD induced obese rats and improved cognitive performance, VD3 supplementation in HFD group also significantly increased GPx and reduced MDA concentrations. Oxidative stress is known to induce apoptosis, which is a key component in neurodegenerative diseases (58). Therefore, anti-oxidants have an essential role in the brain (59).

#### **Recommendation:**

We recommend further studies on the therapeutic effect of vitamin D either by increasing the dose or the duration of the treatment.

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