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Original Research Article

Vitamin D Improves Dementia Induced by Scopolamine in Mice: Histological Study

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Abstract: In Dementia, there is a deterioration in cognitive ability, which affect memory, thinking, and the ability to perform daily activities. Vitamin D is a steroid hormone; its deficiency may play an aggressive role in neurodegeneration disorders. Scopolamine is a muscarinic receptor antagonist that induces memory impairment and oxidative stress. The aim of the work is to determine the histological effect of vitamin D on dementia induced by scopolamine in albino mice. Male albino mice were divided into eight groups of six each. Group 1: Administered T80(1%) for one week, Group 2: Administered scopolamine for one week, Group 3: Administered Vitamin D for one week, Group 4: Administered scopolamine plus Vitamin D for one week (prophylactic effect), Group 5: Administered scopolamine for one week, followed by vitamin D administration for another one week (treatment). Group 6: Administered scopolamine for one week, followed by no treatment for another week, Group 7: Administered donepezil as standard to evaluate learning and memory, Group 8: Administered scopolamine plus Donepezil for one week. Drugs were administered by intraperitoneal route, at the volume of 5ml/kg body weight. All drugs were freshly prepared. The transfer latency was measured using plus maze; mice were killed by neck dislocation; the brains were immediately removed, inserted in 10% formalin, and sent to the histology department, for histological study. Vitamin D repairs the histological damage induced by scopolamine; the improvement induced by vitamin D was much better when given with scopolamine as prophylaxis, more than when it is given after dementia is established as treatment. The model of dementia induced by scopolamine is a reversible model.

Keywords: Dementia, scopolamine, vitamin D, plus-maze, mice, histology.

INTRODUCTION

Dementia is a mental disorder characterized by a progressive deterioration in cognitive ability caused by neurodegeneration (Alzheimer's disease, vascular dementia, and Lewy body dementia) (Prince *et al*., 2013).

Vitamin D is a steroid hormone that has long been known to play roles in calcium homeostasis, bone mineralization, immune cell differentiation, and tumor inhibition (Keeney and Butterfield, 2015). Experimental studies indicate that long-term vitamin D deficiency may play an aggressive role in neurodegeneration involved in Alzheimer's disease (Feart *et al*., 2017).

Scopolamine is a muscarinic receptor antagonist, which can block the cholinergic function of the central nervous system (Liao *et al*., 2020). Scopolamine induces memory impairment and oxidative stress (Yadang *et al*., 2020). It elevates acetylcholinesterase (AChE) levels (Lee *et al*., 2017).

The aim of the work is to find out the histological effect of vitamin D on dementia induced by scopolamine in albino mice.

MATERIALS AND METHODS

Animals

Male albino mice (25-40g) were obtained from animal house of Faculty of Pharmacy, University of Tripoli, they were housed six per cage, in a room under controlled temperature (20 ± °C), humidity (60%), and on 12/12 h light/ dark cycle, with food and water available ad libitum. The animals were kept in the laboratory at least 24 hours before experiments, to adapt to the environment.

Drugs and Chemicals

Scopolamine hydrobromide was obtained from BDH chemicals ltd Poole, England, it was administered at a dose of 6mg/kg (chosen from pilot study). Vitamin D was obtained from Haupt Pharma, France, it was administered at a dose of 10,000 IU/Kg (chosen from pilot study). Donepezil was obtained from Milpharm limited /Aurobind Pharm, Malta., it was administered at a dose of 1mg/kg (Choi *et al*., 2022). Almond oil was obtained from Al-Hermain Products, it was used to dilute and to prepare the appropriate dose of vitamin D (Banafshe *et al*., 2019). Tween 80 (1%) was obtained from Riedel-De Haen AG Seelzf-Hannover, it was injected in volume 5ml/kg and it used as solvent to all drugs except Vitamin D.

Drugs were administered by intraperitoneal (I.P.) route, at volume of 5ml/kg body weight. All drugs were freshly prepared.

Design of the work

The study used 48 male albino mice, the mice were divided into eight groups of six in each. Group 1: Administered T80 (1%) for one week, Group 2: Administered scopolamine (6mg/kg) for one week, Group 3: Administered Vitamin D (10000 IU/kg) for one week, Group 4: Administered scopolamine plus Vitamin D for one week (prophylactic effect of Vitamin D), Group 5: Administered scopolamine for one week, followed by vitamin D administration for another one week (treatment). Group 6: scopolamine for one week, followed by no treatment for another one week, Group 7: As a positive control; Donepezil treated group of a dose 1mg/kg (Choi *et al*., 2022) as standard for plus maze to evaluate learning and memory, Group 8: Administered scopolamine plus Donepezil for one week. The transfer latency (TL) was measured using plus maze.

At the end of administration, mice was killed by neck dislocation; the brains were immediately removed, inserted in 10% formalin and sent to histology department in medical school, for histological study.

Histological Study

Brains of both control and treated male albino mice groups were collected and then fixed in formalin for 24 hours. The specimens were washed twice with 70% alcohol. The fixed tissues were dehydrated in an ascending series of alcohol ranging from 70% to 100% (absolute). The dehydrated tissues were cleared with two changes of Xylene (clearing agent) for 1 hour each. The pieces were then embedded in three changes of molten Paraffin wax at a melting point of 60°C, 1 hour each. Finally, the pieces were embedded in Paraffin blocks. Brains were sectioned on a rotary microtome, sections were 5µm in thickness. , then mounted on glass slides and then deparaffinized in two changes of xylene for 5 minutes each, followed by descending grades of ethanol (absolute, 90%, 80%, and 70%) for two minutes for each. Finally, the prepared sections were stained by routine methods using the Hematoxylin-eosin (H&E) method. The stained sections were examined under a light microscope and the different cell and tissue types were carefully studied and photographed. Brain sections from each study group were evaluated for structural changes, and examined by a histologist and a pathologist. Light microscopy (Leica, Germany) was used for the evaluations (Bancroft and Gamble, 2002).

All relevant ethical guidelines have been followed and any necessary ethics committee approvals have been obtained.

RESULTS

Group I: Control T80, injected with saline (5ml/kg) for one week

The histological evaluation was performed on representative H&E sections from the brain mice group injected with saline T80, revealing normal histological structure for brain parts, cerebral cortex, and hippocampus which shows normal granular and pyramidal layers. Also normal neurons and neuroglia cells (Fig 1, 2, 3, 4 & 5).

Group II: Treated with scopolamine

Histological observations for the brain of H&E stained sections for mice treated with scopolamine 6 mg/kg, revealed mild mononuclear cellular (lymphocytic) infiltration and aggregation, mild vascular proliferation, and perivascular edema. Also, the cerebral cortex shows focal gliosis with microglial proliferation and distributed astrocytes. Some shrunken neurons exhibited eosinophilic cytoplasm and shrinkage of basophilic nuclei. The hippocampus were unremarkable (Fig 6, 7, 8, 9 & 10).

Group III: Treated with vitamin D

Histological observations for brain sections of mice treated with vitamin D, revealed mild gliosis, normal neurons in the cerebral cortex, and unremarkable hippocampus (Fig 11 & 12).

Group IV: Treated with scopolamine + vitamin D

Brain sections showed less mild focal gliosis, vascular proliferation, and mild lymphocytic infiltrate in the cerebral cortex. Also, less edema with some eosinophilic shrunken neurons is seen in the cortex. The hippocampus revealed gliosis of the molecular layer (Fig 13, 14, 15 & 16).

Group V: Treated with scopolamine for one week followed by vitamin D (for another one week)

Brain sections showed normal hippocampus. Focal gliosis and some shrunken eosinophilic neurons exhibit shrinkage basophilic nuclei in the cerebral cortex (Fig 17, 18 & 19).

Group VI: Treated with scopolamine followed by no treatment

Histological observations for brain sections for mice treated with scopolamine followed by no treatment, revealed mild cerebral cortex edema, diffuse gliosis, some vacuolated neurons and few shrunken eosinophilic cytoplasm of the neurons with shrinkage basophilic nuclei. Also, mild perivascular lymphocytic infiltration and aggregation are seen in the cortex. The hippocampus revealed no changes (Fig 20, 21, 22, 23 & 24).

Group X: Treated with scopolamine + donepezil

Histological observations for brain sections of mice treated with scopolamine and donepezil, revealed, mild gliosis and lymphocytic infiltration and aggregation in the cerebral cortex. Normal hippocampus (Fig 25 & 26).

Table 1 and figure 27 showed that brain volumes were not changed after different treatments for one week.

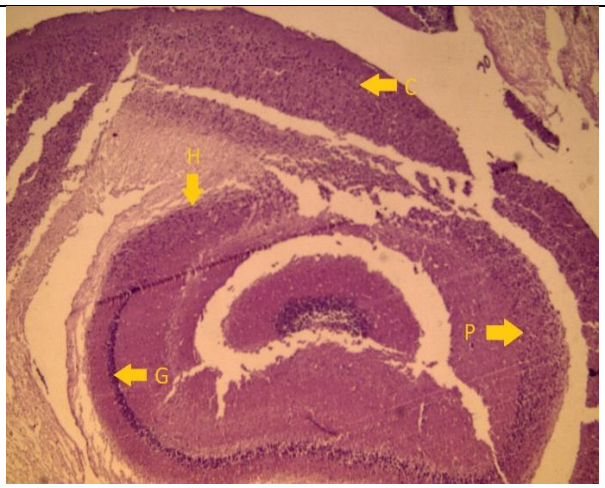

Fig 1: A photomicrograph of group I, Control mouse brain injected with saline T80, showing: normal histological structure for brain parts, cerebral cortex (C) and hippocampus (H) which shows normal granular (G) and pyramidal (P) layers. (H&E. 4X).

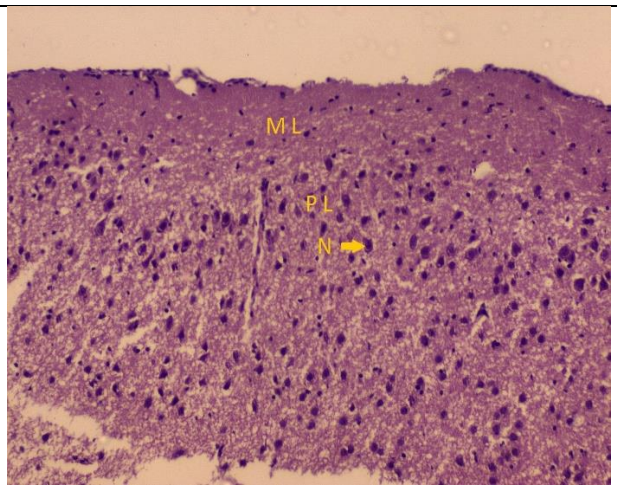

Fig 2: A photomicrograph of group I, Control mouse brain injected with saline T80, showing: Normal histological structure for cerebral cortex including molecular layer (M) and pyramidal layer (P), with normal neurons (N). (H&E. 20X).

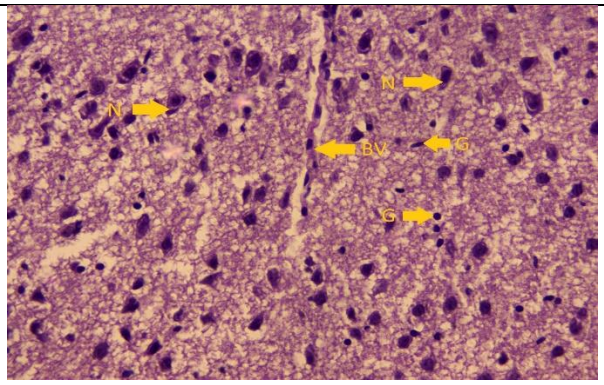

Fig 3: A photomicrograph of group I, Control mouse brain injected with saline T80, showing: normal histological structure for cerebral cortex. Normal neurons (N), blood vessels (BV) and glial cells (G). (H&E. 40X).

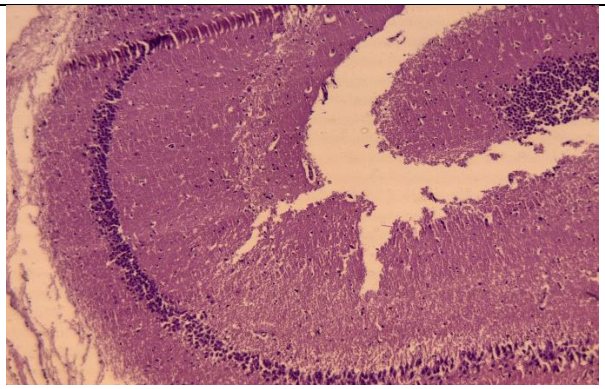

Fig 4: A photomicrograph of group I, Control mouse brain injected with saline T80, showing: unremarkable hippocampus (H&E. 10X).

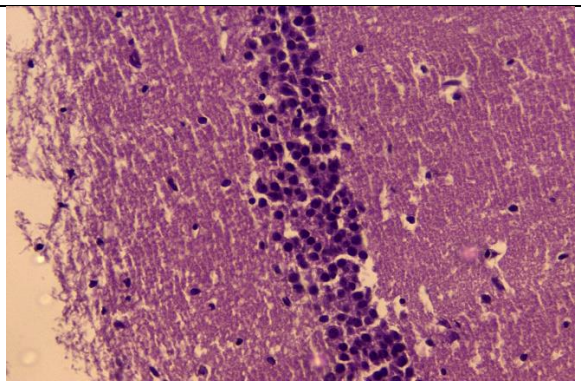

Fig 5: A photomicrograph of group I, Control mouse brain treated with saline T80, showing: unremarkable hippocampus (H&E. 40X).

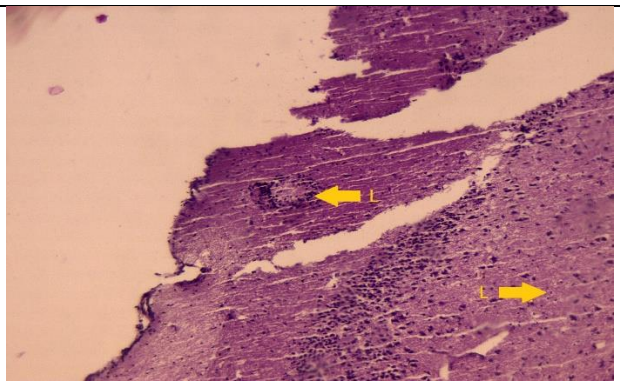

Fig 6: A photomicrograph of group II, mouse brain cerebral cortex, injected with scopolamine 6 mg/kg, showing: mild cellular (lymphocytic) (L) infiltration and perivascular lymphocytic (L) aggregation. (H&E. X10).

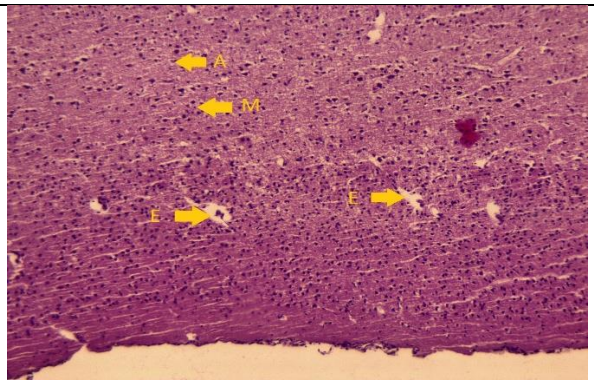

Fig 7: A photomicrograph of group II, mouse brain cerebral cortex injected with scopolamine 6 mg/kg, showing: focal gliosis with microglial (M) proliferation and distributed astrocyte. Vascular proliferation with mild perivascular edema (E). (H&E. X10).

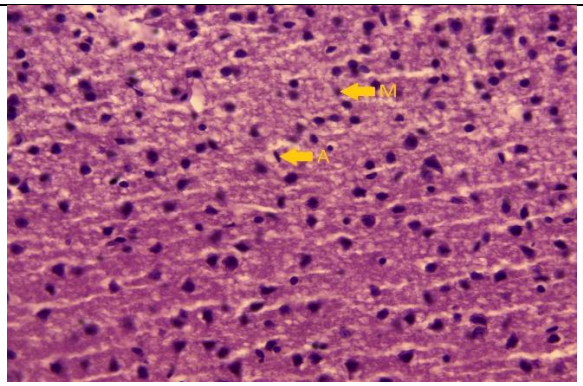

Fig 8: A photomicrograph of Group II, mouse brain cerebral cortex injected with scopolamine 6 mg/kg, showing: focal gliosis including: microglial (M) proliferation and distributed astrocyte (A). (H&E. X40).

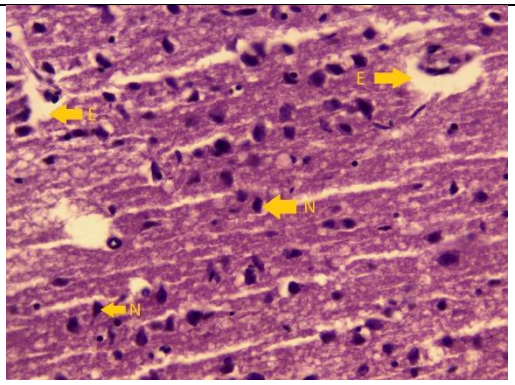

Fig 9: A photomicrograph of Group II, mouse brain cerebral cortex injected with scopolamine 6 mg/kg, showing: perivascular edema (E). Some shrunken eosinophilic neuron (N) exhibited shrinkage basophilic nuclei. (H&E. X100).

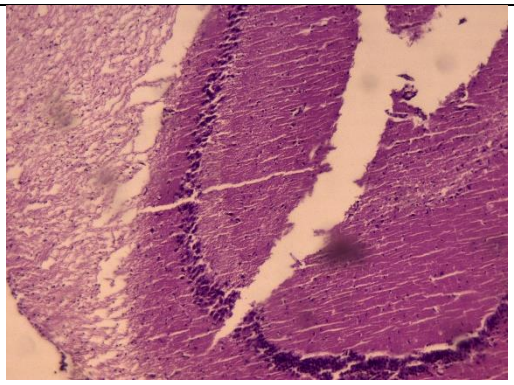

Fig 10: A photomicrograph of Group II, mouse brain hippocampus injected with scopolamine 6 mg/kg, showing: unremarkable hippocampus. (H&E. X10).

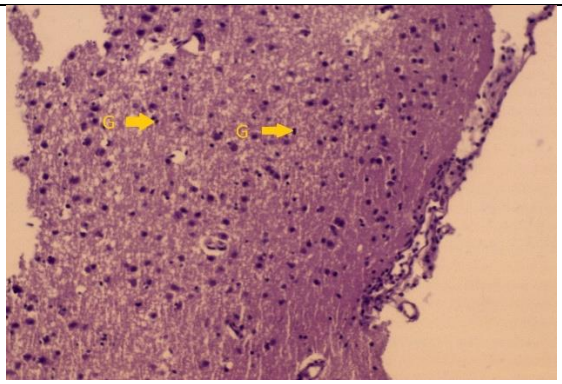

Fig 11: A photomicrograph of Group III, mouse brain cerebral cortex treated with vitamin D, showing: mild gliosis (G). (H&E. X20).

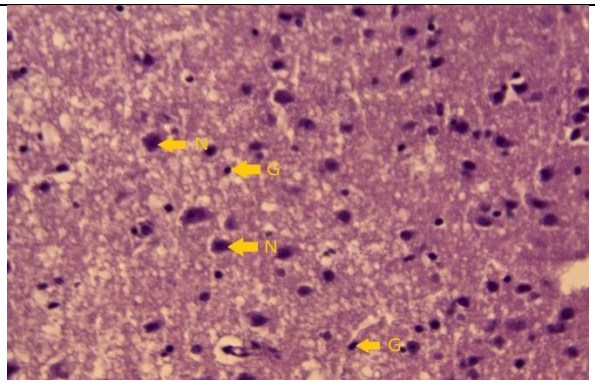

Fig 12: A photomicrograph of Group III, mouse brain cerebral cortex treated with vitamin D, showing: mild gliosis (G) and normal neurons (N). (H&E. X40).

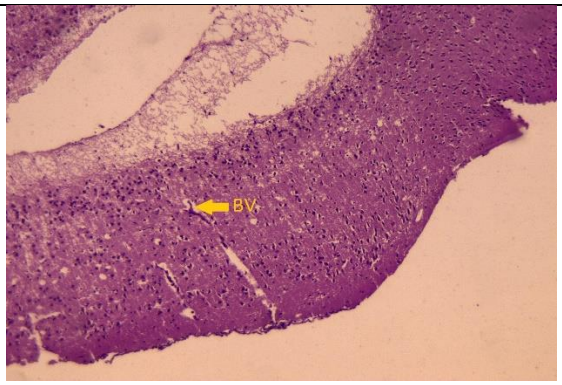

Fig 13: A photomicrograph of Group IV, mouse brain (cerebral cortex) injected with Scopolamine + Vitamin D, Showing: less gliosis and mild vascular proliferation and perivascular edema (BV). (H&E, X10).

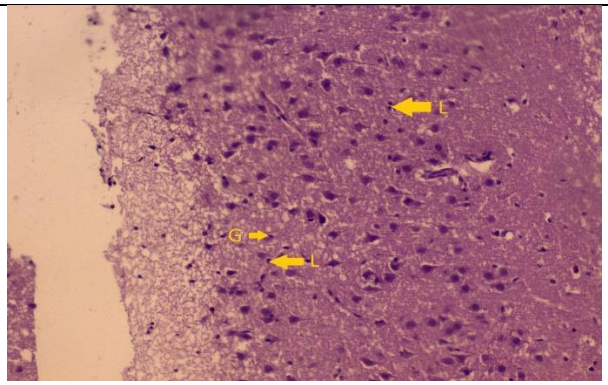

Fig 14: A photomicrograph of Group IV, mouse brain (cerebral cortex) injected with Scopolamine + Vitamin D, Showing: less focal gliosis (G) and mild lymphocytic infiltrate (L). (H&E, X20).

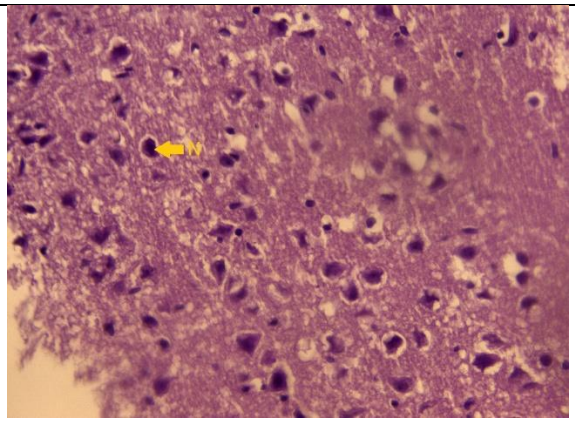

Fig 15: A photomicrograph of Group IV, mouse brain (cerebral cortex) injected with Scopolamine + Vitamin D, Showing: less edema with few eosinophilic shrunken neurons (N). (H&E, X40).

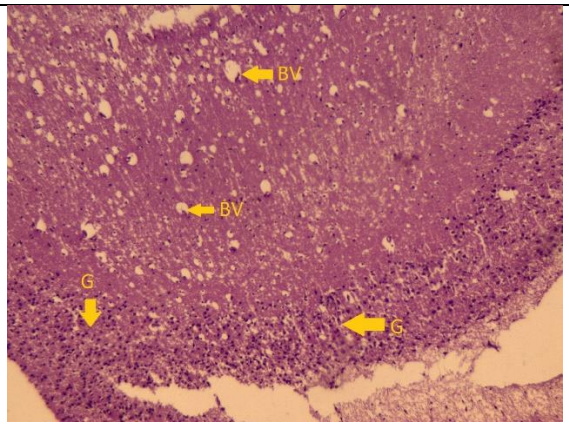

Fig 16: A photomicrograph of Group IV, brain mouse (Hippocampus) injected with scopolamine + Vitamin D. Showing: congested proliferated capillaries blood vessels (BV) and gliosis (G) of molecular layer. (H&E, X10).

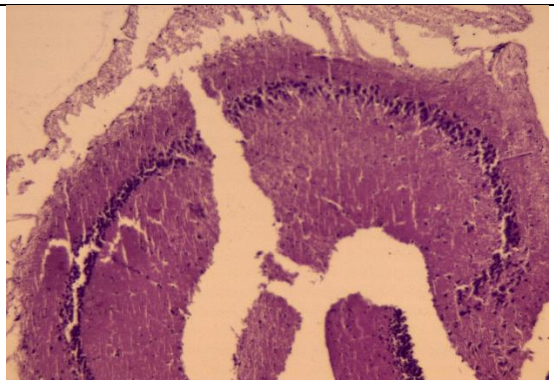

Fig 17: A photomicrograph of Group V, mouse brain treated with Scopolamine followed by vitamin D, showing: normal hippocampus. (H&E, X10).

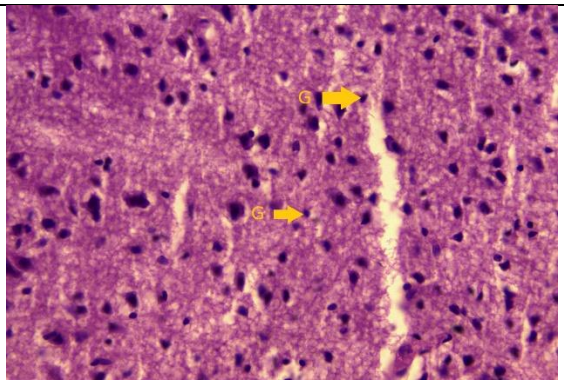

Fig 18: A photomicrograph of Group V, mouse brain treated with Scopolamine followed by vitamin D, showing: Focal gliosis (G) in cerebral cortex. (H&E, X40).

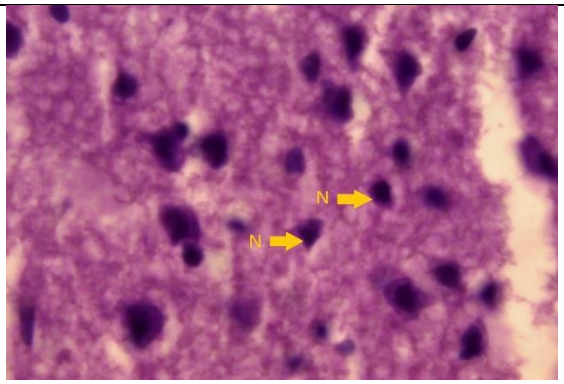

Fig 1: A photomicrograph of Group V, mouse brain treated with Scopolamine followed by vitamin D, showing: some shrunken neurons with eosinophilic cytoplasm and shrinkage basophilic nuclei (N) in the cerebral cortex. (H&E, X100).

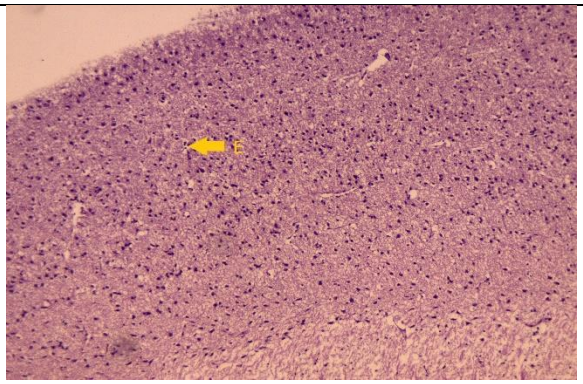

Fig 20: A photomicrograph of Group VI, mouse brain treated with Scopolamine followed by no treatment, showing: diffuse gliosis. (H&E, X10).

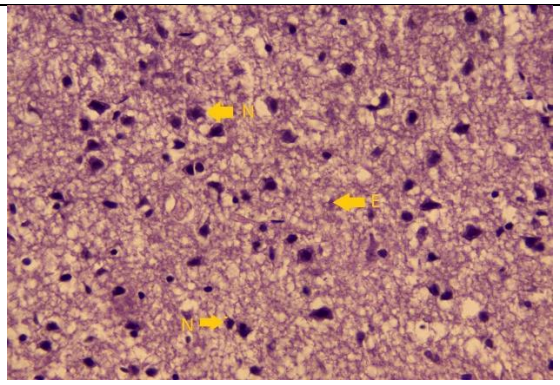

Fig 21: A photomicrograph of Group VI, mouse brain treated with scopolamine followed by no treatment, showing: cerebral cortex edema (E), vacuolated neurons (N) and few shrunken eosinophilic neurons (N) with shrinkage basophilic nuclei. (H&E, X40).

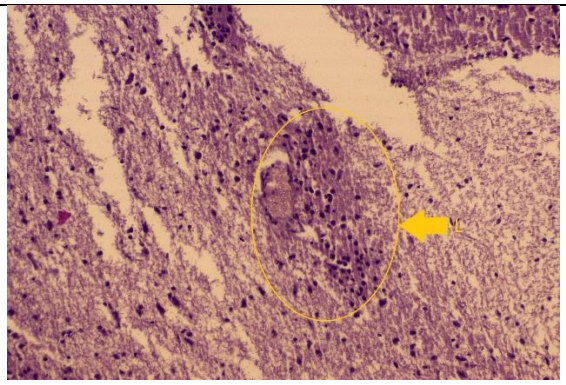

Fig 22: A photomicrograph of Group VI, mouse brain treated with scopolamine followed by no treatment, showing: mild perivascular lymphocytic infiltration and aggregation (L) in cerebral cortex. (H&E, X20).

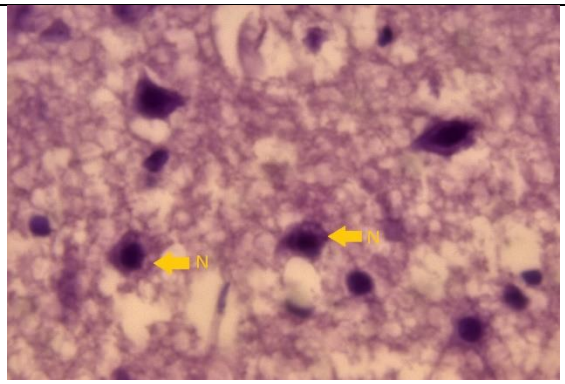

Fig 23: A photomicrograph of Group VI, mouse brain treated with scopolamine followed by no treatment, showing: Cerebral cortex edema and some vacuolated-shrunken neurons. (H&E, X100).

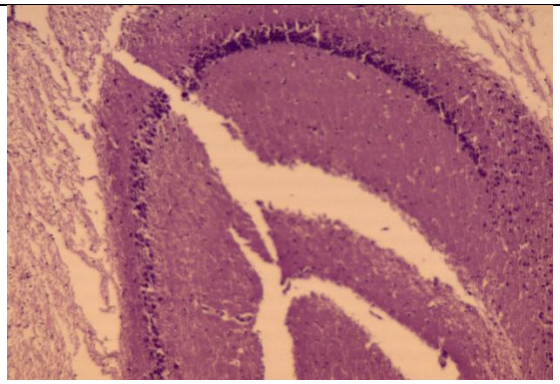

Fig 24: A photomicrograph of Group VI, mouse brain treated with scopolamine followed by no treatment, showing: Normal hippocampus without changes (H&E, X10).

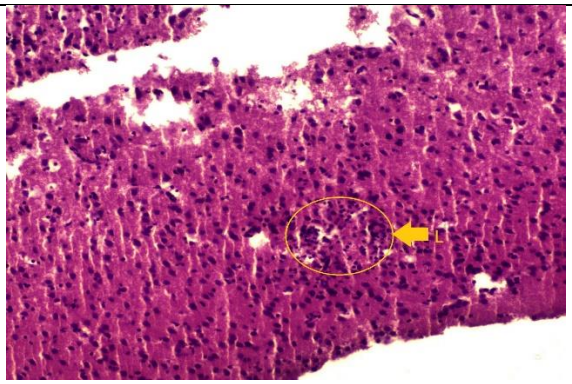

Fig 25: A photomicrograph of Group X, mouse brain sections treated with scopolamine + donpezil, Showed: Mild gliosis and lymphocytic infiltration and aggregation (L) in cerebral cortex. (H&E. X20).

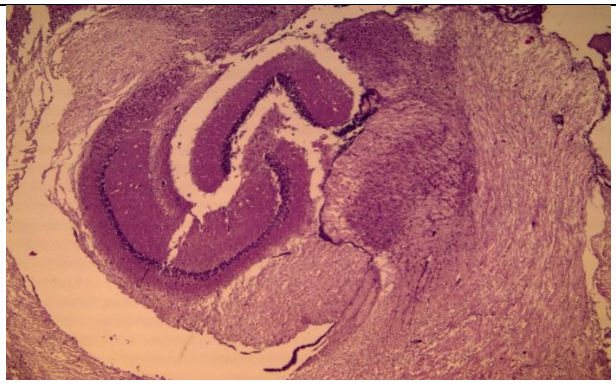

Fig 26: A photomicrograph of Group X, mouse brain sections treated with scopolamine + donpezil, Showed: Normal hippocampus. (H&E. X20).

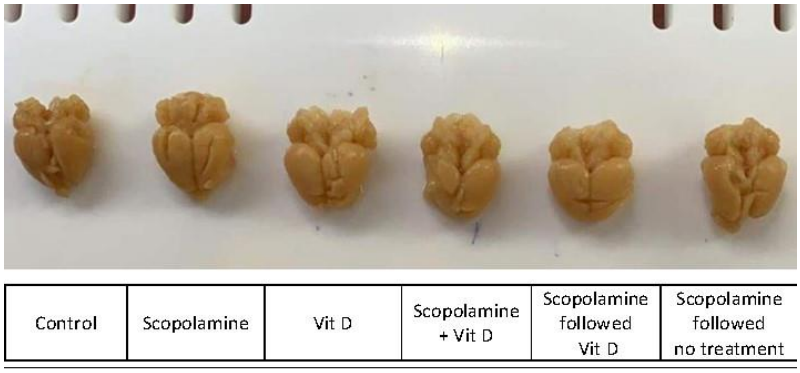

Fig 27: Effect of vitamin D treatment on brain volume after dementia induced by scopolamine

Table 1: Effect of vitamin D treatment on brain width and length after dementia induction by scopolamine

Treatments	Width (mm)	Length (mm)
Control	13	15
Scopolamine	13	15
Vitamin D	13	15
Scopolamine + vitamin D	13	15
Scopolamine followed by vitamin D	13	15
Scopolamine followed by no treatments	13	15
Scopolamine + Donepezil	13	15

DISCUSSION

Dementia describes a collection of symptoms that can affect someone's memory, and their ability to think, process information, and communicate with others (Han and Higuera, 2021). Dementia is a chronic syndrome that leads to a decline in thinking skills, including memory loss and having a hard time with daily activities (Crist, 2022).

Blood brain barrier (BBB) regulates the transport of molecules to the central nervous system and restricts permeability across brain endothelium (Hawkins and Davis, 2005); it is a highly selective semipermeable structural and chemical barrier which prevents foreign objects invading the brain tissue (Cai *et al*., 2018). It prevents toxic compounds from entering from the blood into the CNS, and removes waste products from the brain tissue (Van de Haar *et al*., 2015). It was suggested that blood-brain barrier dysfunction is an early biomarker of human cognitive dysfunction (Nation *et al*., 2019, Lin *et al*., 2021). Blood-brain barrier impairment trigger neuroinflammation and oxidative stress, giving rise to cognitive impairment and the onset of dementia (Cai *et al*., 2018). Evidence indicates that blood–brain barrier impairment play a role in the pathophysiology of cognitive decline and dementia (Van de Haar *et al*., 2015). The production of ROS influences BBB permeability (Pun *et al*., 2009), through the upregulation of cell-disrupting enzymes and protein complexes such as matrix metalloproteinases (MMPs), nuclear factor kappa B (NF-*k*B), VEGF and activator protein 1 (AP-1), among others (Wang *et al*., 2015).

Scopolamine is used to manage and treat postoperative nausea and vomiting and motion sickness (Riad and Hithe, 2022); its treatment induces delirium-like cognitive dysfunction in mice. The cognitive declining properties of scopolamine contributed to its application in neurocognitive research (Cheon *et al*., 2021). Scopolamine administration caused an increase in pro-inflammatory cytokines, and inflammation, leading to memory impairment (Wong-Guerra *et al*., 2017). Scopolamine treatment significantly reduced the expression levels of cholinergic genes (*Chrna3*, *Slc5a7*, *Slc38a5*), which are important for cholinergic communication Cheon *et al*., 2021). Therefore, scopolamine is used, in this study, to induce dementia model using mice.

The present study showed that vitamin D improves dementia induced by scopolamine HBr, either as a prophylactic or treatment. Vitamin D improvement is more powerful when used as prophylactic compared to the treated group.

Vitamin D as a neurosteroid hormone (calcitriol) plays a central role in normal brain functions and the protection of neurons via the maintenance of cellular homeostasis, immune regulation, and modulation of synaptic structure and function; this function is via the VDR (Berridge, 2015). Vitamin D receptors are widespread in brain tissue, and vitamin D's biologically active form (1,25(OH)(2)D3) has neuroprotective effects (Anjum *et al*., 2018). Vitamin D regulates the development and function of nerve cells (Wrzosek *et al*., 2013) and has roles in modulating cognitive processes and in regulating neurotrophic signaling (Dicou 2009, and Koduah *et al*., 2017); evidence point to the neuroprotective role of

vitamin D (Koduah et al., 2017), and also protects from neuroinflammation (Borges *et al*., 2011; Bellia *et al*., 2013). Vitamin D keeps cognitive function in older adults (Lau *et al*., 2017). Calcitriol exerts its steroid-like effects by directly regulating the expression of multiple genes (Eelen *et al*., 2004) through the nuclear vitamin D receptor (VDR) (Eelen *et al*., 2004; Pawel *et al*., 2015).

Vitamin D has neuronal protection through Ca2+ regulation, stimulation of neurotrophin release, interaction with reactive oxygen and nitrogen species, and neurommunomodulatory effects of calcitriol (Kalueff *et al*., 2004; Kalueff and Tuohimaa, 2007), facilitates detoxification through inhibiting the synthesis of inducible nitric oxide synthase (Garcion *et al*., 2002; Chang *et al*., 2004) and increased glutathione levels (Alvarez *et al*., 2014), and enhances neurotrophins synthesis (Peitl *et al*., 2020), enhances antioxidant pathways through induces the expression of several molecules involved in the antioxidant defense system including glutathione, glutathione peroxidase, and superoxide dismutase (SOD) and suppresses the expression of NADPH oxidase (Mokhtari *et al*., 2017).

Vitamin D protects the brain by inducing the production of NGF (Kiraly *et al*., 2006), and reduces inflammatory factors by downregulating proinflammatory cytokines such as serum tumor necrosis factor *α*, IL-10, and IL-6 (Haug et al., 1998; Peterson and Heffernan, 2008). Deficiency of vitamin D was associated with the risk of dementia (Shreeya *et al*., 2022). It was found that cognitive impairment and dementia are caused by a decrease in vitamin D levels (Littlejohns *et al*., 2016). Loss of neuronal excitability occurs with aging and leads to cognitive decline; vitamin D3 has a synaptic role; it has been shown to increase neuronal excitability in the hippocampus of rats (Gold *et al*., 2018). In clinical studies, the cognitive decline in dementia is found to be associated with *brain volume* reductions (Jack *et al*., 2005; Misra *et al*., 2009), even in those in the early stage of dementia (Bumhee *et al*., 2022). A meta-analysis found that lower 25(OH)D levels are associated with lower total brain volume (Navale *et al*., 2022). In this study, the brain volumes were not changed (Table 1, Fig 27); dementia was observed after only scopolamine HBr administration for one week without any change in brain volumes. As mentioned earlier, dementia is a chronic syndrome (Reuben and Epstein-Lubow, 2022); therefore, brain volume changes were not observed in this study.

The *hypothalamus* is important in co-coordinating signals between the nervous system and the endocrine system, via the pituitary gland. Hypothalamic dysfunction can present with dementia (Gottfries *et al*., 1994). The presence of vitamin D receptors in the hypothalamus has suggested a neurosteroid function for active vitamin D, promoting the growth and maturation of neurons (Eyles *et al*., 2005; Annweiler *et al*., 2015). In this study, vitamin D may improve the blood-brain barrier and hypothalamus function, leading to reduce dementia. Circulating 25(OH) vitamin D crosses the blood-brain barrier and enters glial cells and neuronal cells to be converted into the active form of vitamin D (1,25(OH)2D) (Holick, 2015). It was found that $1,25(OH)_2D_3$ reduces the increased production of ROS, and the treatment with $1,25(OH)_2D_3$ prevented BBB disruption through VDR-dependent mechanisms in cerebral endothelial cells (Won et al., 2015). The improvement induced by vitamin D, in this work, may be through the above mentioned different mechanism.

The *cholinergic neurotransmitter* system is responsible for the control of cognitive processes, acquisition, retention of information, and task performance (Cheon *et al*., 2021). Forebrain cholinergic neurons control attention, memory, and cognitive function, and are implicated in cognitive decline and several neurodegenerative diseases (Tata *et al*., 2014; Kilimann *et al*., 2017). It was reported that cholinergic function has a role in age-related memory dysfunction (Altavista *et al*., 1990; Hampel *et al*., 2018). Dementia was induced by scopolamine hydrobromide in mice (Han and Higuera, 2021). The systemic inflammatory responses are under the control of the cholinergic anti-inflammatory pathway through its connections to the vagus nerve (Bonaz *et al*., 2016; Hoover, 2017). Cholinergic signaling can also control peripheral cytokine production by cholinergic anti-inflammatory pathway activity (Rosas-Ballina and Tracey, 2009).

Scopolamine competitively inhibits G-protein coupled post-ganglionic muscarinic receptors for acetylcholine, it is a nonselective muscarinic antagonist; it produces peripheral antimuscarinic properties and central sedative, antiemetic, and amnestic effects (Zhang *et al*., 2017). Scopolamine primarily affects the M1 receptor and has reported H1 receptor activity (Pergolizzi *et al*., 2012; Riad and Hithe, 2023). Scopolamine is capable of producing deficits in learning acquisition, and consolidation (Rahimzadegan and Soodi, 2018). Scopolamine-treated animal models are used in neurocognitive studies; it induces behavioral and molecular features of Alzheimer's disease and other neurocognitive disorders, as impaired cognition, and imbalanced cholinergic transmission in the hippocampus and prefrontal cortex (Ishola, *et al*., 2013; Demirci *et al*., 2017; Wong-Guerra *et al*., 2017). Scopolamine produced damage in learning and short-term memory functions in rodents and humans by disrupting cholinergic transmission (Wong-Guerra *et al*., 2017). Therefore, in this work scopolamine was used to induce dementia through its effect on cholinergic neurons. Vitamin D may improve the dementia through enhancing the prefrontal cortex cholinergic transmission (Alrefaie and Alhayani, 2015; Rodrigues *et al*., 2019).

In the hippocampus, there is a form of neuromodulated synaptic plasticity that depends on the sequential

modulation of two neuromodulators, acetylcholine (ACh) and dopamine (DA) (Brzosko *et al*., 2017). The form of hippocampal Spike-Timing-Dependent-Plasticity (STDP) is sequentially modulated by acetylcholine and dopamine. Acetylcholine facilitates synaptic depression, while dopamine retroactively converts the depression into potentiation (Ang *et al*., 2021). In the CNS, ACh plays a role in synaptic plasticity, including learning ability and short-term memory (Guo *et al*., 2019; Medicine LibreTexts, 2023); where acetylcholine deficiency could be the main cause of impaired plasticity and disrupted learning ability (Sun *et al*., 2022).

Amygdala, frontal lobes, diencephalon and hippocampus are brain region crucial for executive function, memory, attention, and especially for the pathogenesis of delirium. Previous studies showed a significant decrease of neurotransmitters, including dopamine, 3,4-dihydroxy-phenylacetic acid (DOPAC), homovanillic acid (HVA), and acetylcholine, in the amygdala of scopolamine-treated animals (Qiu *et al*., 2016a; Qiu *et al*., 2016b).

Vitamin D is important for brain health; its deficiency may be associated with an increased risk of dementia (Crist, 2022). It was found that vitamin D supplementation increases tyrosine hydroxylase (TH) expression and dopamine production in dopaminergic neurons (Cui *et al*., 2015); vitamin D upregulates N-cadherin which plays a role in the neurogenesis of dopamine neurons, and also improves synaptic plasticity and memory (Schrick *et al*., 2007). In animal (rat) models, it was observed an improvement in cognitive decline with vitamin D supplementation, that mediated by increased choline acetyltransferase activity and a decrease in acetylcholinesterase activity leading to an increase in acetylcholine levels in brain areas (Gold *et al*., 2018). The improvement of dementia by vitamin D, in this study, could as neuromodulator of acetylcholine and dopamine.

Vitamin D upregulated multiple genes which are essential for synaptic plasticity (synaptojanin 1 and synaptotagmin 2, calcium/calmodulin-dependent protein kinase IIδ [CaMKIIδ]), and normal synaptic functioning; this is important for receptors of several neurotransmitters as dopamine, glutamate, and serotonin receptors (Latimer *et al*., 2014). It was observed that vitamin D supplementation increases tyrosine hydroxylase (TH) expression and dopamine production in dopaminergic neurons. The study also shows that vitamin D increases N-cadherin synthesis and upregulation of N-cadherin, which plays a role in the neurogenesis of dopamine neurons as well as in synaptic plasticity and memory (Cui *et al*., 2015). 1,25(OH)D increased expression of TPH2 mRNA (messenger RNA) in the brain, leading to control over serotonin levels in the brain (Kaneko *et al*., 2015). Therefore, 1,25(OH)D treatment prevented both dopamine and serotonin depletion in certain areas of the brain (Cass *et al*., 2006). 1,25(OH)D treatment showed an increase in the activity of choline acetyltransferase and increase acetylcholine levels in brain areas relevant to Alzheimer's Disease (Gold *et al*., 2018). Vitamin D supplementation can prevent the glutamate toxicity implicated in cognitive decline (Annweiler, 2016). Vitamin D may show the improvement of dementia, in this research, through its effect on neurotransmitters important for cognitive and learning function.

In aged adults, there is an increase in the influx of ionized calcium (Ca^{2+}) into neuronal cell bodies, which interferes with the action potentials necessary for long-term potentiation (LTP) and the formation of new memories (Foster, 2007). The increase in Ca^{2+} activates ryanodine receptors (RYRs) and calcineurin, leading to additional release of endogenous calcium and long-term depolarization (LTD); this will cause erasing of memories. The increase in Ca^{2+} is related to increased expression of the L-type voltage-dependent Ca^{2+} channel (LVSCC) (Thibault and Landfield, 996); vitamin D3 downregulates the expression of multiple subunits of LVSCCs, and therefore vitamin D status reduces the major cause of Ca^{2+} dysregulation with aging (Gezen *et al*., 2011). The effects of vitamin D on Ca^{2+} regulation are essential to prevent cognitive decline (Gold *et al*., 2018). Vitamin D has a neuronal calcium regulation effect and protects against excess calcium entry into the brain (Grant, 2009). Vitamin D may improve dementia in this work by downregulation of the expression of multiple subunits of LVSCCs, leading to decrease in calcium entry into the brain.

Studies reported that administration of scopolamine cause an increase in oxidative stress in the brain, especially in the areas associated with memory and learning, such as the hippocampus and prefrontal cortex (Wong-Guerra *et al*., 2017; Ponne *et al*., 2020).

Glutathione protects against glutamate excitotoxicity by acting on glutamate receptors (Limongi *et al*., 2021). Vitamin D supplementation increase the levels of glutathione, and can prevent the glutamate toxicity involved in cognitive decline (Annweiler, 2016); also by upregulating VDR expression (Taniura *et al*., 2006) and exerting antioxidant effects (Ibi *et al*., 2001). The increases in ROS sensitize ryanodine receptors (RYRs), exacerbating the increases in Ca^{2+}, and leading to impairment of memory formation and retention through decreases in the slow component of afterhyperpolarization and long-term depolarization (LTD) (Bodhinathan *et al*., 2010). Glutathione (GSH), the major antioxidant molecule in the brain, is depleted in dementia (Peterson and Heffernan, 2008). The decrease in GSH is associated with enhanced transient receptor potential melastatin type 2 (TRPM2) function in hippocampal pyramidal neurons leading to further Ca^{2+} dysregulation (Riad and Hithe, 2023). Increases in Ca^{2+} increase mitochondrial production of ROS (Sayeed *et al*., 2019). Vitamin D upregulates the expression of glutamate cysteine

ligase (GCL), which is responsible for GSH production, and glutathione reductase (GR), leading to the reduction of glutathione disulfide (GSSG) back to GSH, as a result, the oxidative stress is reduced (Jain and Micinski, 2013; Alvarez *et al*., 2014). In the present study, vitamin D may improve the dementia by its antioxidant mechanism of action.

Neuroinflammation plays a role in reduced cognitive function, and this can turn to dementia that associated with old age (Sartori *et al*., 2012; Mir *et al*., 2021). Vitamin D inhibits the production of nitric oxide by activated microglial cells, further preventing neuronal cell damage (Hur *et al*., 2014). VitaminD3 ($1,25(OH)_2D3$) has a potential immune-modulatory effect by suppressing the expression of several cytokines, including IL-17 (Joshi *et al*., 2011) produced by immune cells (Corrado *et al*., 2022). Vitamin D attenuates the proinflammatory state by increasing IL-10 and decreasing IL-1β expression, which is correlated with decreasing amyloid burden (Briones and Darwish, 2012). 1,25D inhibits the secretion of Th17-related cytokines (IL-17, IFNγ, IL-21, and IL-22) (Ikeda *et al*., 2010; Joshi *et al*., 2011). Conversely, 1,25D promotes the differentiation of regulatory T cells, by inducing the anti-inflammatory cytokine IL-10 and the FoxP3 transcription factor (Urry *et al*., 2012). Data confirmed that $1,25(OH)_2D3$ can exert an inhibitory effect on the production of several pro-fibrotic cytokines, particularly TGFβ, CTGF and FGF2 (Corrado *et al*., 2022).

Transforming growth factor β (TGFβ) is a proinflammatory molecule; and is considered a central player in inflammation (Fiz *et al*., 2021). Vitamin D has differentiating and anti-inflammatory properties (Liu *et al*., 2018; Chen *et al*., 2020); vitamin D was able to refrain the efficacy of TGFβ (Fiz *et al*., 2021). It has been shown that immune cells (monocytes, macrophages, dendritic cells, and lymphocytes) express the vitamin D receptor and vitamin D activating enzyme; this indicates that these cells can produce and respond to activated vitamin D. This suggests that vitamin D low levels may have an impact on inflammatory disorders (Ao *et al.*, 2021).

NLRP3 inflammasome is a multiprotein complex that plays a pivotal role in regulating the innate immune system and inflammatory signaling, leading the release of pro-inflammatory cytokines IL-1β and IL-18 (Hallie *et al*., 2022). The activation of NLRP3 inflammasome causes two main effects, the induction of programmed cell death known as pyroptosis, and/or a pro-inflammatory response caused by the release of inflammatory cytokines IL-1β and IL-18 (He *et al.*, 2016; Hallie *et al*., 2022), accelerating the pathological progression of neurocognitive disease (He *et al*., 2016; Heneka *et al*., 2018). The activation of NLRP3 inflammasome and caspase-1 was observed in three different brain regions, of which the hippocampal area of scopolamine-treated mice showed increased activation (Cheon *et al*., 2021).

Scopolamine treatment increased the level of anxiety and impairments in memory and cognitive function associated with an increase in the level of pro-inflammatory cytokines and NLRP3 inflammasome components; this NLRP3 inflammasome may be the main cause of cognitive impairment after scopolamine treatment Cheon *et al*., 2021). It was found that the increase in inflammation is important in developing neurocognitive disorders, in which increased level of pro-inflammatory cytokines, as TNF-α IL-1β, IL-18, and IL-6, was observed in the brain and blood samples of dementia, and delirium patients (Simone and Tan, 2011; Peng *et al*., 2013).

The pro-inflammatory state increases with age, and this is contributing to cognitive decline and neurodegenerative disorders of the brain (Gold *et al*., 2018). Inflammation is often correlates with the insufficiency of vitamin D, known for its anti-inflammatory properties (Fiz *et al.*, 2021). It has shown that the active form of vitamin D (1,25-dihydroxyvitamin D), has an anti-inflammatory effect (Ao *et al*., 2021; Fiz *et al*., 2021). It was found that vitamin D downregulates proinflammatory cytokines such as serum tumor necrosis factor α, and IL-6 (Peterson and Heffernan, 2008; Zittermann *et al*., 2008), reduces biomarkers of inflammation such as C-reactive protein (Zhou and Hyppönen, 2023), and attenuates neuroinflammatory age-related changes (Moore *et al*., 2007).

Active vitamin D may act as a neuroprotectant through the suppression of excess inflammatory neurovascular damage caused by proinflammatory cytokines and attenuation of amyloid proteins, commonly observed in Alzheimer disease (Gold *et al*., 2018). Activated vitamin D suppress dendritic cell cytokine production, specifically, interleukin (IL)-12, which affects the differentiation of T helper cells into Th1 cells, and IL-23, and also affects the differentiation of T helper cells into Th17 cells. Vitamin D also promotes expression of the anti-inflammatory cytokine IL-10 (Tomoka *et al*., 2021); and prevents the increase of autoimmune response (Tomoka *et al*., 2021).

It was demonstrated the beneficial effect of vitamin D in minimizing the risk of developing autoimmune diseases; this effect was through the inhibition of the synthesis of various pro-inflammatory Th1, Th9 and Th22 cytokines and stimulates the synthesis of anti-inflammatory Th2 cytokines (Sîrbe *et al*., 2022). The active form of vitamin D reduces Th1-type differentiation and the secretion of inflammatory cytokines (IL-2, IFNγ, and TNF-α), and promotes Th2-type differentiation and the secretion of anti-inflammatory cytokines (IL-4, IL-5, and IL-10) (Ao *et al*., 2021). Vitamin D in this study may improve the dementia through its anti-inflammatory and immune modulatory effects.

Oxidative stress may cause neurocognitive disease (Cheon *et al*., 2021). Oxidative stress induces apoptosis,

which is a key component in neurodegenerative diseases (Johnson *et al*., 2012). Oxidative stress markers have been found to be associated with dementia (Hatanaka *et al*., 2015). Therefore, antioxidants have an essential role in the brain where high oxygen consumption produces free radicals, e.g., reactive oxygen species (ROS) (Lobo *et al*., 2010).

$1,25(OH)_2D_3$ showed a protection against cerebral endothelial dysfunction by inhibiting ROS production and NF-*k*B activation, this may explain the neuroprotective effects of vitamin D hormone (Won *et al*., 2015). It was found that vitamin D enhances neuronal survival by regulating neurotrophic factor-3 (NT-3) and Glial-derived neurotrophic factor (GDNF) synthesis in an animal model of AD (Fernandes de Abreu *et al*., 2009).

Vitamin D receptor (VDR) has a negative regulator of TGF-β/Smad signaling; therefore, impaired VDR signaling and reduced expression of VDR with decreased levels of its ligand may thus contribute to hyperactive TGF-β signaling (Pawel *et al*., 2015).

The inflammatory cytokine (Fiz *et al*., 2021), Transforming growth factor β (TGFβ) has a crucial activity in the regulation of inflammatory responses (Nolte M, Margadant, 2020). TGFβ-mediated ROS production, it is responsible for redox imbalance and oxidative stress (Abe *et al*., 2013; Krstić *et al*., 2015); it promotes the production of reactive oxygen or nitrogen species (ROS/RNS) higher than in physiologic conditions, which leads to redox imbalance and apoptosis (Yan *et al*., 2014).

It has been reported that $1,25(OH)_2D3$ reduces the synthesis and expression of TGFβ (Corrado *et al*., 2022). A study detected that the effects of TGFβ are reverted by the vitamin D signaling pathway (Ricca *et al*., 2019). It was demonstrated that TGFβ is able to induce the expression of VDR, while VDR exerts negative feedback on TGFβ signaling by inhibiting TGFβ-mediated metabolic effects. Activation of the VDR signaling pathway triggered by TGFβ suppresses mitochondrial energy metabolism, and ROS production (Fiz *et al*., 2021).

Many genes responsible for redox homeostasis are under the control of vitamin D and its receptor combined with retinoid X receptor (VDR/RXR) (Barsony and Prufer, 2002). Vitamin D controls the expression of the redox-sensitive transcription factor, nuclear factor erythroid 2-related factor 2 (TNrf2); TNrf2 is activated in response to increased levels of ROS (Berridge, 2015). Vitamin D regulation of klotho expression, an antiaging gene, also reduces oxidative stress (Wang and Sun, 2009; Berridge, 2015).

The nerve growth factor is an essential molecule for hippocampal and cortical neurons' neuronal survival. A study conducted by Gezen-AK *et al*., (2014) showed that vitamin D regulates the expression and release of nerve growth factor (NGF). Vitamin D significantly increases the NGF release, and the protective effect of vitamin D against cytotoxicity was observed. In this work, vitamin D improve the dementia through its regulation of different growth factors related to learning and memory decline.

In this study, Vitamin D improves dementia, much better when is given with scopolamine (prophylaxis), more than when it is given after dementia is established (treatment). Interestingly, a study found that vitamin D lost its efficacy when added after prolonged incubation with TGFβ, which agreed with the conclusions that the beneficial power of vitamin D is related to the early stages of TGFβ-driven transition (Fiz *et al*., 2021).

It was that found vitamin D/VDR regulates estrogen receptor 1 (ESR1) and insulin growth factor 1 (IGF1); these two pathways prevent brain aging and neurodegenerative disease through neuronal survival, synaptic plasticity, and against Aβ toxicity (Landel *et al*., 2016). 1,25-(OH)2D3 increases estrogen receptor (ER) expression (Katzburg *et al*., 2004), and is an important factor in estrogen biosynthesis (Kinuta *et al*., 2000). Investigations suggested that the levels of estrogen receptors play an important role in neuroprotection and against neuroinflammation-induced degeneration in AD (Liu and Zhao, 2013; Lan *et al*., 2015). Vitamin D reverses insulin signaling disruption in a model of AD (Gold *et al*., 2018), through the regulation of klotho expression (Forster *et al*., 2011); where klotho (antiaging gene) is responsible for inhibiting the insulin/IGF1 signaling pathway, thereby preventing aging (Delcroix *et al*., 2018).

It was found that the genes related to vitamin D status and normal functions of vitamin D have been involved in cognitive decline (Kueider *et al*., 2016). Certain single nucleotide polymorphisms (SNPs) in the gene for vitamin D binding protein (VDBP) showed a correlation with both lower serum vitamin D levels and cognitive decline with age (Kueider *et al*., 2016).

In addition to the genetic impacts of vitamin D/VDR, vitamin D also acts via the membrane-associated rapid response steroid-binding receptor (MARRS). In mice, activation of MARRS was shown to induce cognitive improvements and increased axon density in the medial prefrontal and perirhinal cortices, inducing cognitive improvements (Tohda *et al*., 2013). The dementia improvement observed in this work by vitamin D, could be through the

regulation of growth factors, hormones and many genes responsible for redox homeostasis.

CONCLUSION

Vitamin D improves the histological damage induced by scopolamine; the improvement induced by vitamin D was much better when it is given with scopolamine as prophylaxis, more than when it is given after dementia is established (treatment). The model of dementia induced by scopolamine is a reversible model.

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