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Neuroprotective Effects of Curcumin and Vitamin D3 on Scopolamine-Induced Learning-Impaired Rat Model of Alzheimer's Disease

Saima Khan and Kaneez Fatima Shad

Abstract

The purpose of this study was to find out the beneficial effects of curcumin and vitamin D3 in rats treated with scopolamine as to generate animal model of tauopathies, i.e., neurodegenerative disorders, including Alzheimer's disease (AD). Abnormal phosphorylation of tau results in the transformation of normal adult tau into paired-helical-filament (PHF) tau and neurofibrillary tangles (NFTs). Our results indicated that scopolamine-treated rats exhibit increased levels of hyperphosphorylated tau protein along with PHF, and curcumin and vitamin D3 lowered the levels of PHF better than donepezil. The effect of abnormal hyperphosphorylation of tau was also detected in the hematoxylin and eosin staining of brain tissues as well as in the western blot analyses in our experimental rat models of AD. This abnormal level of hyperphosphorylated tau probably causes cognitive and memory deficit as observed in different behavioral tests on exploratory groups. Hyperphosphorylated tau may have disrupted the microtubule network in experimental rats. Signs of temporal region dementia noted during behavioral studies may be linked to the neurodegeneration and abnormal hyperphosphorylation of tau observed in our experimental animal model of AD. The curcumin and vitamin D3-treated group presented lower levels of hyperphosphorylated tau and a better behavioral response. Thus, inhibition of abnormal hyperphosphorylation of tau offers a promising therapeutic target for AD and related tauopathies.

Keywords: Alzheimer's disease, memory impairment, inflammation, scopolamine, curcumin, vitamin D3, donepezil

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder, which present mainly in the elderly patients. It is characterized by progressive loss of cognitive functions, amyloid β ($A\beta$) deposition, and formation of paired-helical-filament (PHF) tau and neurofibrillary tangles (NFTs) in the brain cells. NFTs are formed inside the cell bodies of the neurons. These NFTs cause shrinkage of neurons and resultant loss of cognition and learning [1, 2].

Tau protein is a highly soluble microtubule-associated protein found abundantly in the neuronal cells of the central nervous system (CNS). Tau proteins are the product of alternative splicing from a single gene, designated for microtubule-associated protein tau (MAPT) in humans, located on chromosome 17. There are six isoforms of tau found in the human brain. They can be distinguished by their binding domains. Tau has 79 potential phosphorylation sites on the longest isoform [3]. Tau is the major microtubule-associated protein of a mature neuron. The other two neuronal MAPs are MAP1 and MAP2, which are involved in tubulin interaction and promotion of its assembly into microtubules and stabilization of the microtubule network [4].

Normal adult human brain contains 2–3 moles of phosphorylated tau protein. Hyperphosphorylation of tau decreases its normal function. In Alzheimer's disease, brain tau is approximately three- to fourfold more hyperphosphorylated than the normal adult brain. This hyperphosphorylated state polymerized into paired-helical-filament tau and when mixed with straight filaments (SF) formed neurofibrillary tangles (NFT). The hyperphosphorylated tau in AD brain has the ability to sequester normal tau, MAP1, and MAP2, to disrupt microtubules, and to self-assemble into PHF/SF. Abnormal hyperphosphorylated tau, in the cytosol, does not polymerized into PHF [5]. The cytosolic hyperphosphorylated tau is involved in tubulin assembly but inhibiting its normal assembly and disrupting microtubule [6]. In addition, with hyperphosphorylation of tau, conformational changes and abnormal cleavage of tau may contribute to the pathogenesis of AD [7, 8]. Tau hyperphosphorylation has been reported in AD and other tauopathies; thus, the inhibition of abnormal hyperphosphorylation of tau offers a promising therapeutic target for AD and related tauopathies [9].

Similarly, oxidative stress is also strongly linked to neuronal dysfunction and neuronal cell death [10]. It is suggested that oxidative stress plays a significant role in the pathological conditions of AD by enhancing A β deposition, tau phosphorylation, and loss of synapses and neurons [11].

Reactive oxygen species (ROS) are by-products of biochemical and physiological processes in the body and can cause oxidative damage to macromolecules in an uncontrolled manner that may lead to many chronic diseases. Thus, overproduction of ROS is a hallmark of neurodegenerative disorders and other diseases [12, 13].

Neuroinflammation also causes neurodegeneration in the vulnerable regions of the brain such as the hippocampus. Microglia and astrocytes play important roles in neuroinflammation and contribute to neurological disorders [14, 15].

Previous studies showed that curcumin acts as an antioxidant by activating macrophages to remove ROS-like, superoxide anions, H₂O₂, and nitrite radicals. Its anti-inflammatory properties were tested in vivo and in vitro on animals in acute and chronic inflammatory conditions [16]. Moreover, vitamin D also reported to play a part in the cerebral processes of detoxification by interacting with reactive oxygen and nitrogen species in the rat brain and by regulating the activity of glutamyl transpeptidase [17, 18], which is a key enzyme in the metabolism of glutathione. Vitamin D₃ is the active form of vitamin D. This study investigated the effects of curcumin and vitamin D₃ on memory and learning, by assessing the behavioral responses of scopolamine-induced learning-impaired rats through assays involving the locomotive and maze activities and histological and protein analysis in the rat brain tissues. The findings of this study show that inducing learning impairment in rats by using scopolamine followed by treatment with curcumin and vitamin D₃ results in neuroprotection and attenuation of cognitive deficits as shown by reduced brain tissue damage in histoanalysis, decreased accumulation of abnormal proteins with immunoblot analysis and increased in the numbers of correct responses to behavioral stimuli during locomotive and maze tests.

2. Literature review

Aging is the primary risk factor for AD development. Aged population is prone to oxidative stress that results in the degeneration of their brains [19, 20]. Diets containing saturated fat and less intake of vitamin E and C are linked with the risk of AD [20]. AD patients suffer from memory impairment along with other cognitive deficits such as language, visuospatial skills, insight, and apraxia. Most patients may suffer from other symptoms such as depression, hallucination, apathy, and delusions at later stages of AD [21]. Numerous studies have indicated that accumulation of amyloid beta proteins ($A\beta$) and phosphorylated tau (p-tau) are the key pathological hallmarks of AD [22]. Similarly, oxidative stress changes ionic homeostasis and other biochemical parameters, which ultimately causes neuronal dysfunction and cell death leading to progressive dementia associated with extensive $A\beta$ and tau pathology [23]. Tau is a neuronal microtubule-associated protein that is responsible for maintaining the microtubule dynamics and its function of transportation by axons and neurite outgrowth [24]. Animal models have demonstrated that loss of synaptic plasticity is one of the key components in the neurodegenerative process of AD, and tau is one of the contributing factors for neurodegeneration [25]. Literature indicated that the oxidative stress plays a significant role in the pathology of AD by enhancing $A\beta$ deposition, tau phosphorylation, and loss of synapses and neurons [26].

Vitamin D is a group of fat-soluble secosteroids that helps to absorb calcium, magnesium, phosphate, iron, and zinc. Vitamin D protects the brain from the degenerative processes of AD by binding itself with vitamin D receptors [27]. Vitamin D deficiency has been associated with neurological and psychiatric disorders. Previous studies revealed that it controls Ca^{2+} homeostasis in the hippocampus by regulating intracellular Ca^{2+} . It also controls neurotrophic agents and protects the brain from $A\beta$ -42 accumulation by stimulating phagocytosis. It also protects acetylcholine deficiency by increasing the activity of choline acetyltransferase in the brain. Due to its multiple biological targets, vitamin D can be used as an aide with the standard anti-dementia treatment. Among vitamin Ds, the most important compound is vitamin D3, also known as cholecalciferol. Increasing evidence highlights the impact of vitamin D deficiency as an important factor in various central or peripheral neurological diseases, especially multiple sclerosis and other neurodegenerative diseases, such as amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease [28].

Curcumin (*Curcuma longa* (Haldi)) was used as a treatment in the animal models of AD. It was observed that curcumin reduced the formation of NFTs, $A\beta$ deposition, and $A\beta$ oligomerization. Curcumin can cross the blood-brain barrier because of its lipophilic nature. It can also inhibit acetylcholinesterase (AChE) activities [29] and can bind with the plaques leading to the alleviation of behavioral impairment [30–31]. Curcumin also acts as an antioxidant by activating macrophages to remove ROS-like radicals, superoxide anions, H_2O_2 , and nitrite radicals [32–35].

Donepezil is available with the trade name "Aricept" developed by Eisai Inc. in 1983. It is a reversible AChE inhibitor, used for the treatment of mild to moderate dementia in AD patients. It has a long plasma half-life of 70 h. It is a noncompetitive reversible inhibitor of AChE that improves the function of cholinergic transmission. It increases the concentration of acetylcholine by preventing its hydrolysis. Animal studies have shown its selectivity for brain tissues and inhibition of AChE activities in smooth, striated, cardiac muscles. It can also inhibit AChE in red blood cells similar to its effect at synapses in CNS. AChE inhibition in red blood cells has been used as an indicator of the clinical effectiveness of donepezil in Alzheimer's disease patients [36].

Scopolamine is a tropane alkaloid that acts as a muscarinic receptor antagonist. Scopolamine is used to study memory and cognition in animal model of AD. Studies have shown that scopolamine provides a suitable pharmacological model of memory defect. Scopolamine administration characterizes cognitive deficits resulting in the impairment of verbal learning, spatial learning, and reaction time [37]. Scopolamine can also have an influence on other neurotransmitter systems due to the functional interaction of cholinergic neurons with other neurotransmitter systems [38]. Cholinergic transmission is blocked, resulting in cognitive impairment in a rat model of AD [39]. Histological studies of the brain of Alzheimer's patients have revealed the presence of activated microglia and reactive astrocytes around the A β plaques. The chronic activation of microglia secretes cytokines and some reactive substances that exacerbate A β pathology; thus, neuroglia plays an important part in the pathogenesis of AD [40]. Curcumin has a lipophilic property that is capable of passing through all cell membranes and thus exerts its intracellular effects. Curcumin has antiproliferative actions on microglia. A minimal dose of curcumin affects the neuroglial proliferation and differentiation. The overall effect of curcumin on neuroglial cells involves decreased astrocytes proliferation, improved myelogenesis, and increased activity and differentiation of oligodendrocytes [40].

3. Aims and objectives of this study

This study was conducted with the following objectives:

1. To determine the effects of curcumin, vitamin D3, and donepezil on behavioral responses of scopolamine-induced memory and learning-impaired rats.
2. To examine the structure of brain tissues obtained from scopolamine-treated rats with and without curcumin or vitamin D3 or donepezil.
3. To investigate the concentration of hyperphosphorylated tau protein in scopolamine-treated rat brain tissues with donepezil, curcumin, or vitamin D3.

4. Material and methods

4.1 Animals

Male Sprague Dawley rats of 200 ± 25 g were obtained from the animal house (PAPRSB Institute of Health Sciences Animal Facility, University Brunei Darussalam). Thirty animals were divided into five groups of six animals per group and reared under a standard laboratory condition with free access to food and water. Rats were acclimatized in a laboratory condition for a minimum of 1 week before undergoing behavioral test. The food was restricted under a daily feeding regime to maintain the weight of the rats.

All experiments were performed during daylight for 27 days, and all groups except group I (saline control) received daily scopolamine injection (2.5 mg/kg) to induce excitotoxicity. Curcumin, vitamin D3, and donepezil were administered to rats orally (**Table 1**). All experiments were conducted in accordance with institutional ethics guidelines for animal care and use (**Table 2**).

Group	Treatment
Group 1	Saline control (0.9% saline)
Group 2	Scopolamine (2.5 mg/kg) injection
Group 3	Scopolamine (2.5 mg/kg) injection and curcumin (80 mg/kg) oral
Group 4	Scopolamine (2.5 mg/kg) injection and donepezil (2.5 mg/kg) oral
Group 5	Scopolamine (2.5 mg/kg) injection and vitamin D3 (0.0179 mg/kg) oral

Table 1.
List of treatments received by each of the five groups of rats.

	Control	Scopolamine	Curcumin	Vitamin D3	Donepezil
Mean	17.42607	176.1054	27.29171	33.8713571	34.12914
SEM	1.261983	71.34413	14.49811	11.5630538	12.12356

Table 2.
Data expressed as mean ± standard error of the mean (SEM), expressed as the mean of time taken by different groups to reach the reward chamber each alternate day for 27 days.

4.2 Experimental design

4.2.1 Behavioral tests

4.2.1.1 Rectangular maze

This test was used to investigate learning and memory. The maze consisted of a rectangular box with an entry and a reward chamber with food, which were placed at the opposite ends of the box (**Figure 1**). All groups were given training in rectangular maze 1 week before drug administration. Each animal was placed in the same spot, recording the time taken by the animal to reach the reward chamber (transfer latency). Five readings were taken for each animal, and the average was calculated as their learning score [41–43].

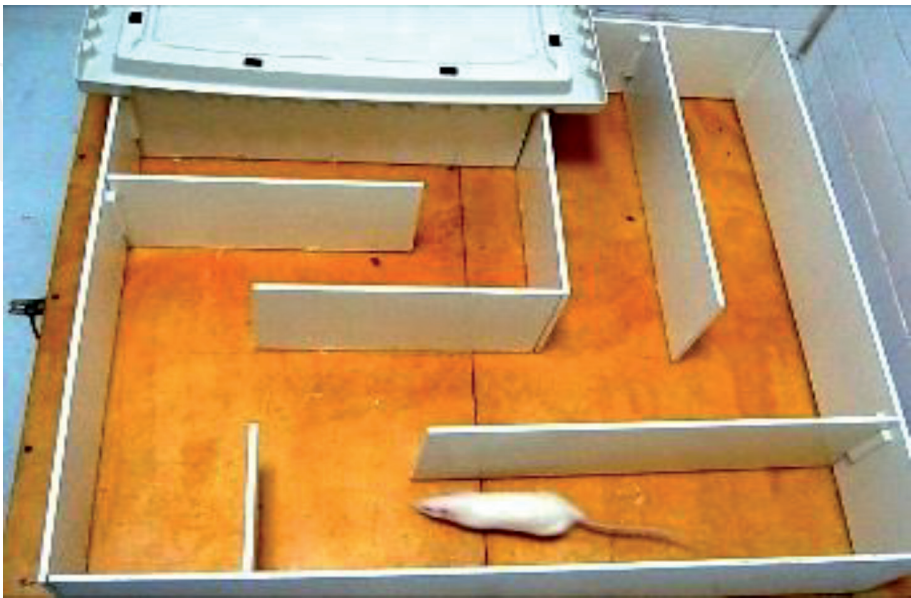


Figure 1.
Rectangular maze test.

4.2.1.2 Locomotor activity

Actophotometer was used to measure the locomotor activity (**Figure 2**). Each animal was treated with their respective compound, was placed in actophotometer, and was given 2 min in activity cage. When the beam of light falling on photocell was cut off due to the movement of the animal, an activity count was recorded. The increase or decrease in locomotor activity was then calculated [42, 43].

4.2.2 Histology

After the behavioral study was conducted, the animals were anesthetized, and their brains were removed and stored in 4% paraformaldehyde (**Figure 3**). The brains were embedded in paraffin and kept in the refrigerator. Paraffin sections (5 μ m) were prepared using rotary microtome (**Figure 4**) and stained with hematoxylin and eosin [44]. Photographs were taken for each section.

4.2.3 Estimation of protein concentration

4.2.3.1 Immunoblotting

Curcumin, donepezil, and vitamin D3 reduce tau phosphorylation in the brains of a scopolamine-treated rat model of Alzheimer's disease.

The brain tissues were dissected from the coronal area with clean tools and put on ice as quickly as possible to prevent protein degradation by proteases. The tissues

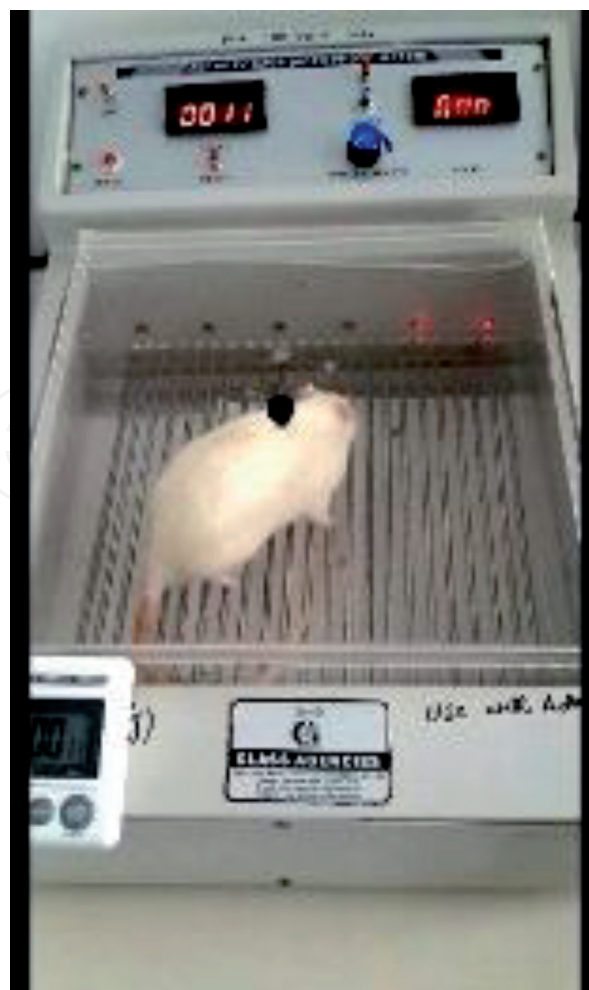


Figure 2.
Actophotometer for locomotor activity.

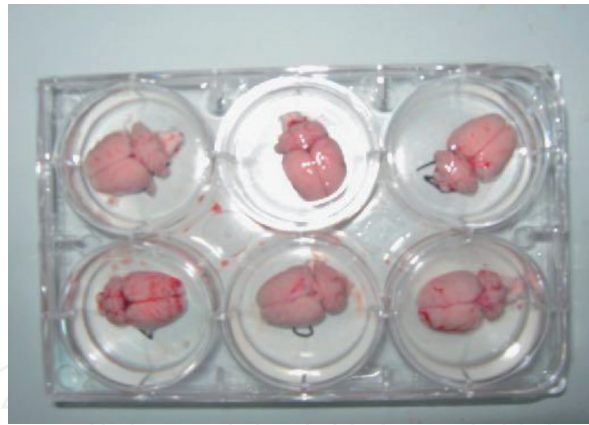


Figure 3.
Freshly dissected rat brains.



Figure 4.
Paraffin embedding for immunohistochemistry.

were placed in microcentrifuge tubes and immersed in liquid nitrogen to snap-freeze. They were homogenized on ice after adding 1× ice-cold lysis buffer, rinsed twice with the same buffer, and agitated on a shaker for 2 h at 4°C. After centrifugation for 20 min at 12,000 rpm at 4°C, the supernatant was transferred into a fresh tube kept on ice discarding the pellet. A small volume of lysate was sampled to perform a protein quantification assay.

After boiling each cell lysate in Tris-buffered saline, 0.1% Tween 20 (TBST) at 100°C for 5 min, 50 µg of protein was loaded into the wells of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel for immunoblot analysis. After gel running for 1–2 h at 100 V, the proteins were then transferred onto the membrane and blocked for 1 h at room temperature. The membrane was then incubated with 1:1000 dilution of primary antibody in blocking buffer followed by washing three times with TBST for 5 min each wash. The membrane was incubated with the 1:1000 dilution of conjugated secondary antibody in blocking buffer at room temperature for 1 h and washed three times with TBST at 5 min each wash. Excess reagents were removed, and the membrane was covered with transparent plastic wrap. The image was acquired using the darkroom development techniques for chemiluminescence detection.

4.3 Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). The analysis of variance (ANOVA, single factor) was used to measure transfer latency with statistical significance set at $p < 0.05$.

5. Results

5.1 Rectangular maze test

The effects of curcumin and vitamin D3 on scopolamine-induced rats were investigated using the rectangular maze test comparing the results obtained with that of donepezil, a widely accepted AD standard drug. Rats that were injected with scopolamine showed significantly higher transfer latency, indicating the longer time for rats to reach the food (nearly 200 s). Also, there was no sign of improvement during the successive days (**Figure 5**). Rats treated with curcumin and vitamin D3 displayed significant reduction in transfer latency, which means that treated rats did not take a longer time to reach the food, less than 50 s as shown in **Figure 6**. Furthermore, there was a slight reduction in the latency time from day to day. The effect of these two compounds was also comparable to that of donepezil.

5.2 Locomotor activity

Actophotometer was used to measure locomotor activity by counting total photocell counts per rat for 2 min. Rat injected with scopolamine showed progressive decline in their locomotor activity (**Figure 7**), and average values were shown in **Table 3** and **Figure 8**. Rats treated with curcumin and vitamin D3 showed an initial increase in locomotor activities then slightly declined after 7 days followed by leveling off in the succeeding days. In contrast, rats treated with curcumin and vitamin D3 showed high locomotor activity compared with the non-treated control rats treated with donepezil that exhibited similar response as those treated with curcumin and vitamin D3, suggesting that these two compounds had triggered alertness and excitatory activities in scopolamine-treated rats.

5.3 Histology

Non-treated rats injected with scopolamine revealed prominent degeneration of cells and decrease number of nuclei in their brain tissues as compared to those treated with curcumin and vitamin D3. Moreover, treatment with curcumin, vitamin D3, and donepezil showed similar cell morphology similar to the control group demonstrating brain cells that appeared normal (**Figure 9**).

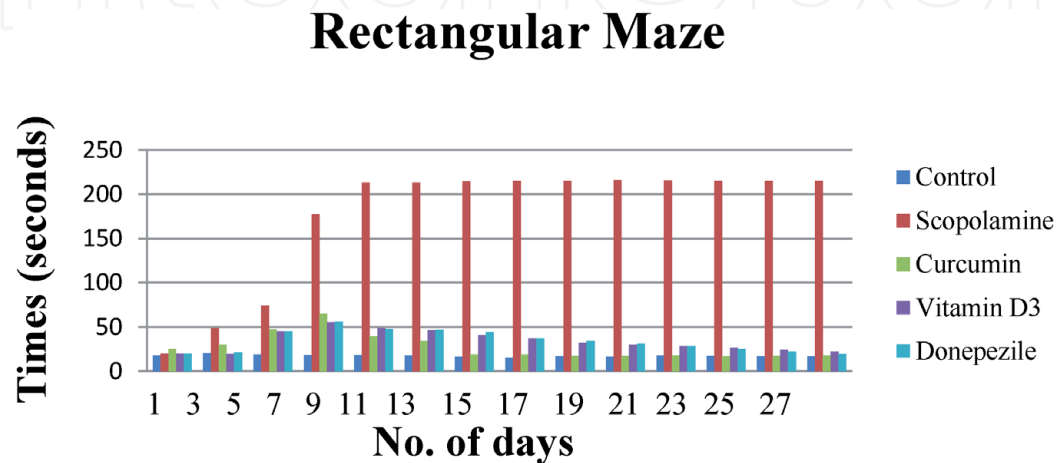


Figure 5. Effect of curcumin, vitamin D3, and donepezil on latency time compared with the disease control group (mean, n = 6). The histogram shows the mean of latency time in seconds.

Rectangular Maze

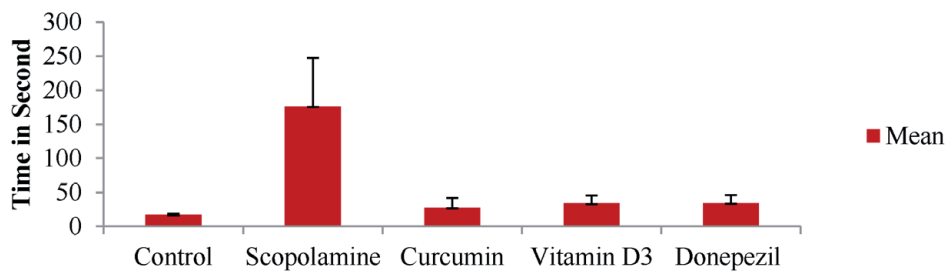


Figure 6. Time taken to reach the reward chamber in the rectangular maze. Y-axis represents time in second. Data expressed as mean \pm SEM.

Locomotor Activities

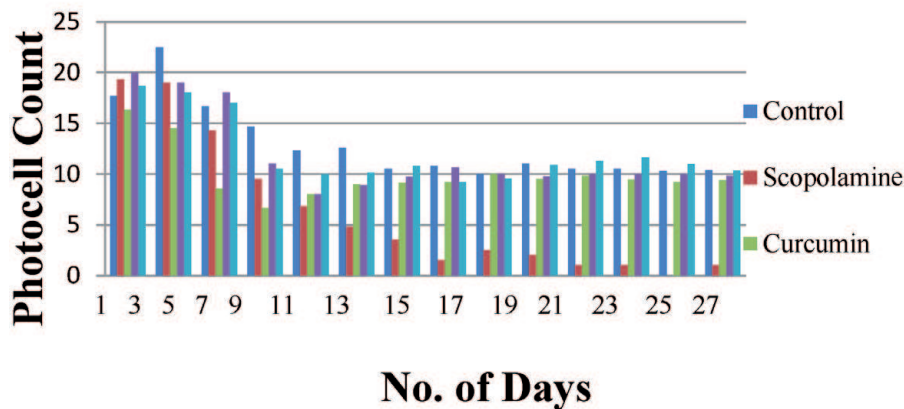


Figure 7. Effect of curcumin, vitamin D3, and donepezil on latency time compared to scopolamine-treated group (mean, $n = 6$). Graph shows mean latency time in seconds.

	Control	Scopolamine	Curcumin	Vitamin D3	Donepezil
Mean	12.88686	6.161643	9.911429	11.77929	12.0664286
SEM	3.712271	6.766518	2.502838	3.992999	3.23978538

Table 3. Data expressed as mean \pm SEM for the total number of photocell counts for each group.

Locomotor Activity

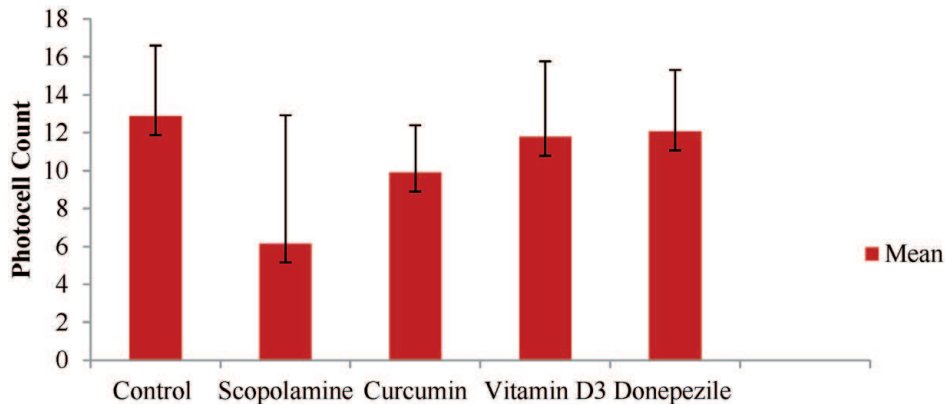


Figure 8. Locomotor activities of all rats except scopolamine-treated rats in actophotometer showed no significant difference. Data expressed as mean of photocell count (mean \pm SEM, $n = 6$) of animals on each alternate day for 27 days.

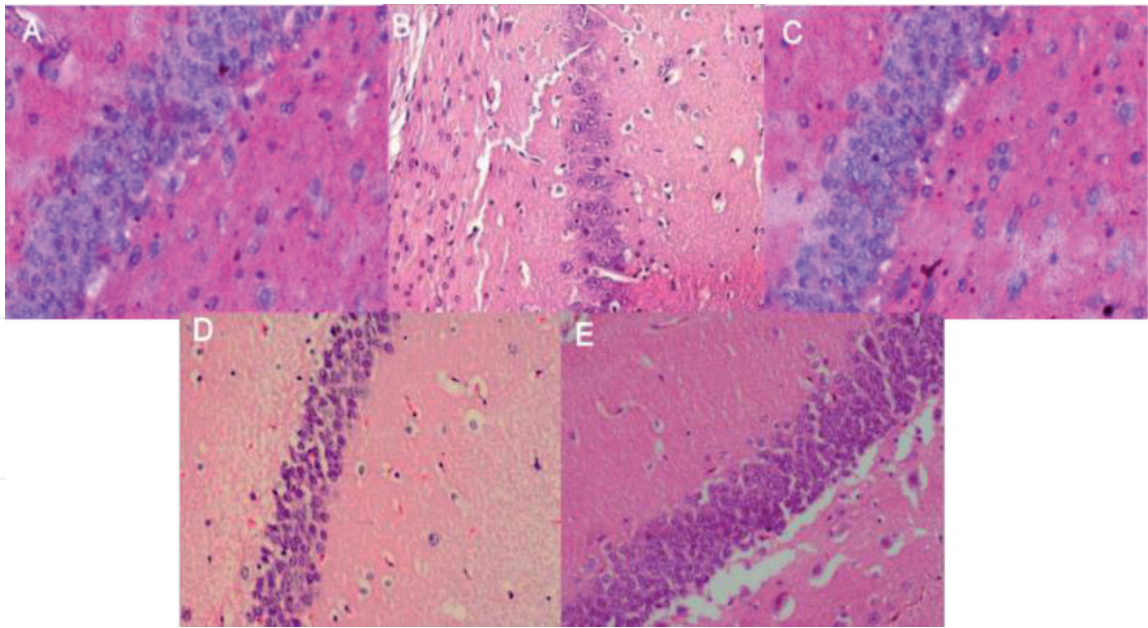


Figure 9. Hematoxylin and eosin staining of rat brain tissues: A, control; B, scopolamine-induced; C, curcumin-treated; D, vitamin D₃-treated; and E, donepezil. The images show no significant difference in the cellular histology of the hippocampal area (cornu ammonis) (CA₃) in the experimental groups (curcumin, vitamin D₃, and donepezil) as compared with those of scopolamine group, which showed less number of nuclei stained as revealed by H and E staining. Arrows in scopolamine slide B indicated the gaps around the neuronal cells of coronal sections (5 μ m) at magnification 40 \times .

5.3.1 Immunoblotting

See **Figure 10**.

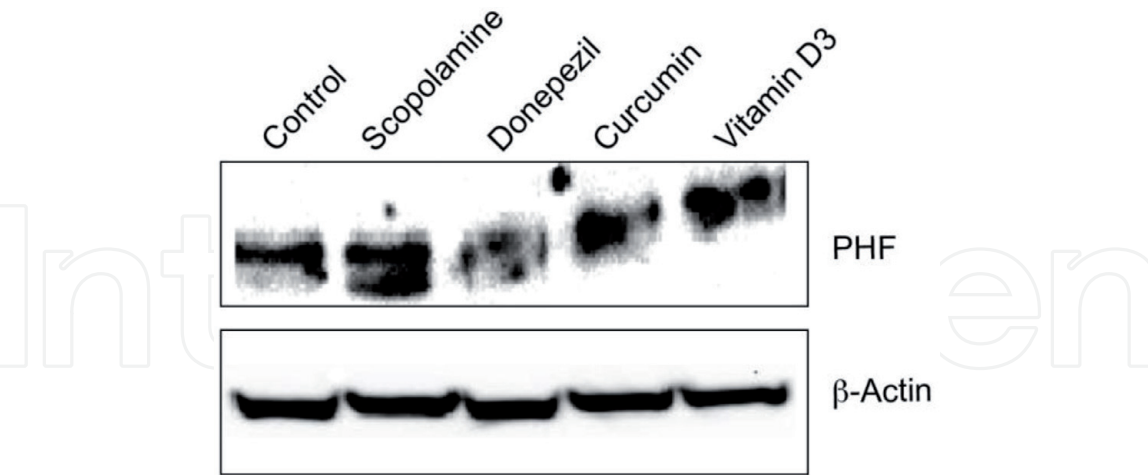


Figure 10. Western blot analyses of scopolamine-treated and other treatments (curcumin and vitamin D₃ and donepezil) groups showing difference in the levels of hyperphosphorylated tau in a rat model of AD. (a) Immunoblot of hippocampus homogenates from treated rats (scopolamine, vehicle, treated with curcumin and vitamin D₃) using the PHF monoclonal antibodies and (b) normalized with β actin.

5.3.2 Vitamin D₃ + scopolamine

See **Tables 4, 5 and Figure 11**.

S/No.	Area	Percent
1	13690.4	17.975
2	22076.4	28.986
3	16481.8	21.641
4	10,292	13.513
5	13,621	17.884

Table 4.
Densitometry data were obtained using image J software, exhibiting relative density of protein from all groups.

S/No.	Area	Percent
1	4240.34	23.631
2	3502.99	19.522
3	1857.51	10.352
4	4913.31	27.381
5	3429.87	19.114

Table 5.
Densitometry data were obtained using image J software. Representing relative density of Tau protein for all groups.

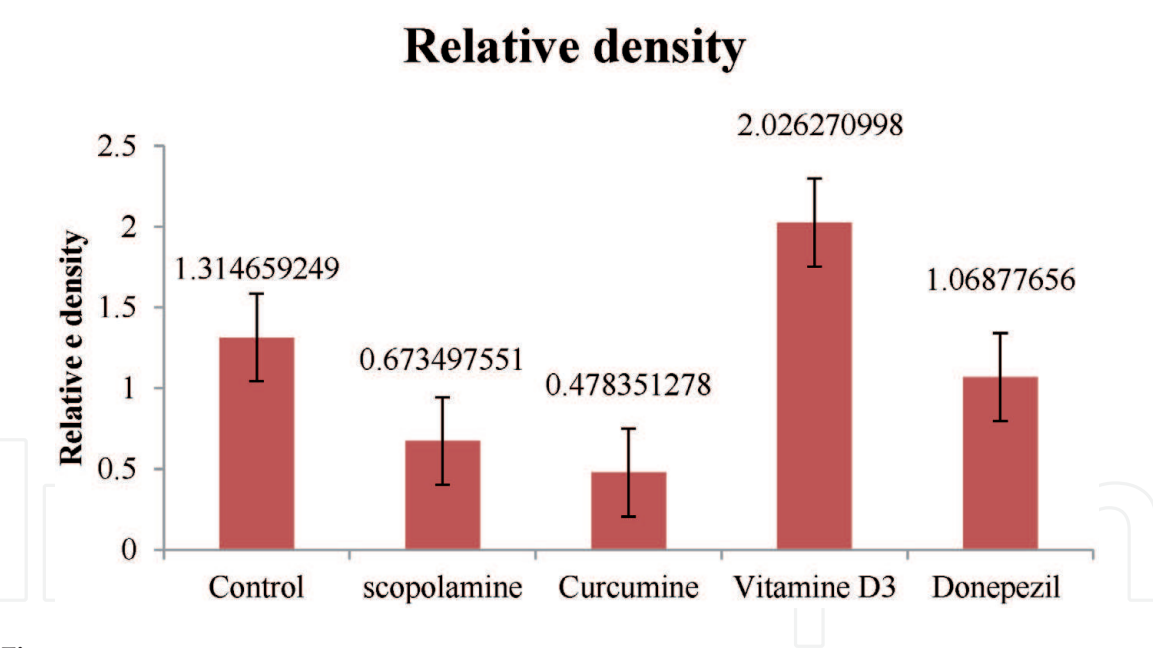


Figure 11.
Densitometry data obtained from image J software, presented as relative density of tau protein present in all groups.

6. Discussion

This study investigated the effects of curcumin and vitamin D3 on learning and memory and locomotion. The first part of the study involved subjecting the rats to several behavioral tests and examining their memory competencies and locomotor responses. Histological studies were also done on rats' brains to observe the changes that have occurred in the brain tissues after various treatments. The results obtained from rats treated with curcumin and vitamin D3 were compared with

donepezil-treated rats. Scopolamine (muscarinic cholinergic antagonist) was used to induce memory impairment in rats [45]. Curcumin was selected as the previous research showed that curcumin could be used to recover learning and memory abilities in rats in AD and other inflammatory conditions [46]. Literature also reported that curcumin facilitates learning and memory functions by diminishing or preventing lipid peroxidation in the brains of aged rats [47]. In general, curcumin is a well-known oxygen free radical scavenger [46].

Vitamin D plays an important role in the regulation of numerous neurotransmitters including acetylcholine, dopamine, serotonin, and gamma aminobutyric acid. Several studies have also been reported that vitamin D deficiency is associated with neurological dysfunction and that supplementation of vitamin D may induce a protective effect against neurological disorders [48]. Based on the results of this study, the rats that were injected with scopolamine only revealed a gradual increase in the latency time until day 9, indicating a longer time required for rats to reach the end of the maze where food as a source of attractive stimuli was placed. After the ninth day, the latency time remained high and was three times higher than that of curcumin, vitamin D3, and donepezil. Rats treated with curcumin and vitamin D3 exhibits reduced latency time. A slight increase in time was observed between days 1 and 7 and gradually decreased up to day 27. The daily decrease in the latency time represented the effects of these two compounds on long-term memory. When comparing between curcumin and vitamin D3, rats treated with curcumin had slightly lower latency time than vitamin D3, suggesting that curcumin was comparatively more effective than vitamin D3 and donepezil in improving learning and memory among rats. Rats treated with donepezil initially showed low latency time, but remained constant until the 27 days. The similarity of latency time values obtained among curcumin, vitamin D3, donepezil, and the control suggested that curcumin and vitamin D3 have comparable effects like that of donepezil and may reverse the memory impairment induced by scopolamine.

The locomotor activity of rats was investigated by placing each rat in an actophotometer for 2 min and then assessing their movement as compared with those treated with curcumin and vitamin D3. Furthermore, the results indicated a decline in daily activities suggesting signs of slowing down. Vitamin D3 showed an increase in locomotor activity, which was comparable with those of donepezil and the controls confirming previous studies on the role of vitamin D in motor activities [49].

Curcumin exhibited slightly less action when compared to vitamin D3, donepezil treated, and control rats but was still exhibiting higher movements than scopolamine only. After 9 days, the locomotor activity for each treatment except scopolamine became relatively stable throughout 27 days and did not show any signs of slowing down, indicating that rats treated with vitamin D3 and curcumin exhibited signs of alertness that continued for a longer time.

The effects of each treatment were also histologically examined in rat brains. Sections of the brain tissue from the region of hippocampus were stained to investigate histological appearance before and after the treatment with selected compound. The cells in the brain tissue treated only with scopolamine exhibited less number of nuclei that appeared to be shrunken and smaller than with those of the control group. Treatment with curcumin and vitamin D3 showed no difference as compared with those of the brain tissue treated with donepezil and control group, suggesting that the brain tissues seemed to have recovered after the rats were treated with curcumin and vitamin D3. The difference in the levels of tau protein was also assessed using immunoblotting. In scopolamine-induced group, phosphorylated tau proteins were relatively higher than other groups indicating a state of proliferation in the brain tissues. Previous studies reported that accumulation of phosphorylated tau protein is one of the hallmarks of AD [50].

Western blot images were also assessed visually by making comparisons between bands in different lanes (**Figure 10**). Densitometry data obtained from image J software presented as relative density of tau protein found in all groups (**Tables 4 and 5**). After the rats were treated with curcumin and vitamin D3, the levels of tau proteins were reduced suggesting an attenuation of phosphorylated tau proteins in the rat brains, confirming the earlier studies (**Figures 10 and 11**) [51, 52].

7. Conclusion

We concluded that Alzheimer's disease is a progressive neurodegenerative disorder characterized by gradual memory loss and shrinkage of neuronal cells particularly in the hippocampus and basal forebrain regions. Curcumin and vitamin D3 have biomedical qualities that protect the brain from degeneration associated with AD. In this study, the behavioral tasks involving rectangular maze test and locomotor activity were used to determine if curcumin and vitamin D3 could improve learning and memory among rats subjected to scopolamine-induced impaired cognition. With cognitive impairment, the correct response rate of animals during acquisition and retention period was significantly lower than that of the control group. However, treatment with curcumin and vitamin D3 has increased their correct response rate for both tasks that became equal with those of the control group ($p < 0.05$). Tissue analysis by H and E staining of the rat brain from the scopolamine group showed less number of cells, which was improved upon the treatment with curcumin and vitamin D3, resulting in significantly increase in the number of cells with no gap around them. This was accompanied by reduced level of abnormal tau proteins detected via immunoblot analysis. Together, these findings demonstrate that curcumin and vitamin D3 have the potential to reverse some cognitive deficits, correct memory impairment, and protect the brain from degeneration.

The animal model of AD has shown improvement in learning and memory after exposure to curcumin and vitamin D3 treatment, which slowed down the progress of AD pathologies delaying the onset of AD. With potential as a treatment for AD in future, the active structure and the target of both curcumin and vitamin D3 can be further investigated to elucidate the molecular mechanism by which their beneficial effects can be enhanced for the improvement of AD patients. Vitamin D due to its multiple biological targets can be used as an adjunct to standard anti-dementia treatment in AD. Curcumin has intensively been studied for the improvement of AD symptoms, and existing investigations on inhalable curcumin and ar-turmerone on neural stem cells (NSCs) are currently under clinical trials.

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