Original Article

The Effect of Simvastatin and Vitamin D Co-Administration on Rats Brain Function and Behavior: A Behavioral and Biochemical Study

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Abstract

Background & Objective: The simvastatin (Sim) is a lipophilic statin and can cross the blood-brain barrier. The role of vitamin D (Vit D) in brain development and function has been supported over the past decade. This study aimed to evaluate the effect of simvastatin on memory and anxiety levels in healthy male rats.
Materials & Methods: In this experimental study, 36 male Wistar rats weighing 250-300 g were randomly divided into six groups (n=6) including control, Vit D (5 µg/kg/day; IP), Simvastatin (1 mg/kg/day; orally) (SimL), Simvastatin (10 mg/kg/day; orally) (SimH), SimL + Vit D and SimH + Vit D. After 28 days, at the end of the treatment, the behavioral anxiety test and memory behavioral test were performed. Then, the rats were euthanized, and oxidative stress markers of the brain, serum low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and cholesterol levels were investigated. Data were analyzed by SPSS 24 software and Tukey's test.
Results: Co-administration of simvastatin and vitamin D significantly increased working memory, catalase activity, total antioxidant capacity, HDL-C, and decreased anxiety levels, malondialdehyde (MDA) concentration, cholesterol, and LDL-C compared to the control group ($p<0.05$). However, the administration of simvastatin and vitamin D alone did not significantly change the mentioned parameters compared to the control group ($p>0.05$).
Conclusion: Co-administration of simvastatin and vitamin D can improve brain function by reducing oxidative stress and cholesterol levels.

Keywords: Anxiety, Simvastatin, Vitamin D, Working memory, Rat

Introduction

The hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are known as statins. Statins have been approved by the US Food and Drug Administration (FDA) as cholesterol-lowering drugs. These drugs target the liver and inhibit cholesterol biosynthesis, which reduces the amount of intracellular cholesterol in liver cells and then regulates the number of Low-Density Lipoproteins (LDL-C) receptors on the cell surface (1). Statins are generally accepted as a treatment of choice for the prevention and treatment of cardiovascular disease (2). Fat reducing agents such as statins are among the most widely used drugs in a country like Canada. In fact, between 2007 and 2011, more than 2.9 million people in Canada took fat-reducing drugs (3).

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The results of research on laboratory animals and humans indicate that simvastatin can easily affect the brain due to its lipophilic and ability to cross the blood-brain barrier (4). Different types of statins cross the blood-brain barrier with various efficiencies, depending on the degree of being lipophilic (5). The lipophilic statins such as simvastatin affect the nervous system and improve the concussion treatment, improve learning in the rat, increase protein synthesis and cell differentiation into mature neurons in mice, and increase BDNF and VEGF expression (6). Simvastatin is known to possess antioxidant properties in addition to its cholesterol-lowering effects (7). In this regard, the results of studies showed a reduction in anxiety associated with the use of simvastatin (8). Over the past decade, there have been reports of adverse cognitive effects of statins in some cases such as memory loss and amnesia (9). The US FDA announced that "memory loss" and "confusion" were among the cognitive effects reported in patients treated with statins (10). However, the results of some studies indicate the positive effects of statins on cognitive function and memory in laboratory animals and humans (11, 12).

Vitamin D is a fat-soluble vitamin that is synthesized in the skin with exposure to sunlight or is ingested from dietary supplements or food. After hydroxylation in the liver into 25-hydroxyvitamin D, the active metabolite can enter the cell, bind to the vitamin D-receptor, and subsequently to a responsive gene such as calcium-binding protein (13). Recent studies have shown that low serum 25–hydroxyvitamin D are associated with depressive symptoms (14). Some statins decrease 25-hydroxyvitamin D, but some statins increase their level, for example, rosuvastatin increases its level, while fluvastatin does not have a significant effect on increasing the level of 25-hydroxyvitamin D (15). Studies have shown that statins lower plasma cholesterol levels, however, cholesterol is required for vitamin D synthesis. There is a lot of debate about vitamin D reduction due to statins and their effect on public health (16).

Therefore, this study aimed to determine the effect of simvastatin in the presence of vitamin D supplementation on working memory, anxiety levels, cholesterol level, and oxidative stress of healthy male rats.

Materials & Methods

Animals and treatment

In this experimental study, 36 male adult Westar rats weighing 250±20 g were purchased from authorized laboratory animal breeding center (Laboratory Animal House, Urmia University, Urmia, Iran). Rats were kept in cages under standard conditions of temperature (22.00 ± 2.00 ˚C), relative humidity (50.00 ± 10.00%), and light (12 hr light/dark), fed with a standard pellet diet and had free access to water. The experimental protocol and procedures complied with international guidelines for care and use of laboratory animals and were approved by the Urmia University (No: IR-UU-AEC-3/1033 / DA). Following two weeks, the rats were divided into six groups (n=6), including control, Vit D (5 µg/kg/day; IP) (17), Simvastatin (1 mg/kg/day; orally) (Low dose of Simvastatin; SimL) (18), Simvastatin (10 mg/kg/day; orally) (High doses of Simvastatin; SimH) (19), SimL + Vit D and SimH + Vit D. The duration of this study was considered as 28 days. The chemicals were purchased from Sigma (St. Louis, USA) unless otherwise stated. The Vit D and Simvastatin were dissolved in sesame oil (Barij Essence, Tehran, Iran) and distilled water, respectively.

Working memory measurement

After 28 days, at the end of the treatment, the evaluation of the working memory was performed in the cross-Maze. Cross-maze is made of wood and has 4 equal arms measured 13×50×10 cm connected to a circular center plate in the middle of the maze. The arms are named A, B, C, and D. To perform the test, each rat was placed in the central maze area, allowing free access to all areas of the maze over 10 minutes. The number and sequence of animals entering the arms were recorded as one of the letters A, B, C, and D. Periodic behavior was considered to be the successive and consecutive inputs to all arms in the quadrant series. Thus,

the percentage of rotation was calculated from the ratio of the number of actual rotations observed to the number of possible rotations (Total number of arms entering - 3) × 100.

$$Alternation\ percent = \frac{The\ number\ of\ actual\ alternations}{The\ number\ of\ possible\ alternations} \times 100$$

$$Possible\ alternations = Total\ number\ of\ entries - 3$$

Anxiety measurement

An elevated plus maze (EMP) was used for measurement of anxiety (20). This assessment is based on two instincts: one is the sense of rodent search and the other is the avoidance of open and bright environments. In this way, the animals are more inclined to spend their time in closed arms. An anxiety testing device is a wooden instrument that has four arms in the form of a plus-Maze. During the 5-minute period that the animal moved freely in different parts of the maze, four factors were measured by observation: the number of times the animal entered the open arm, the number of times the animal entered the closed arm, the length of time the animal stays in the open arm, the length of time the animal stays in the closed arm.

$$\text{Percentage of time spent in the open arm} = \frac{\text{Time to stay in the open arm}}{\text{time to stay in the open + closed arms}} \times 100$$

Sampling

After behavioral measurement, the rats were euthanized with ketamine (75.00 mg kg^{-1}, Alfasan, Woerden, The Netherlands) and xylazine (10.00 mg kg^{-1}; Alfasan, Woerden, The Netherlands), both intraperitoneally (IP). Then, blood samples were taken from the heart of the animals. After one hour, all blood samples were centrifuged at 1500 rpm for 10 minutes to isolate their serum. The obtained serum was carefully separated by a sampler and stored in Ependrov microtubes in a freezer at -80 ° C for serological assessments. The brain tissue was carefully removed and transferred to a −80°C freezer for evaluation of oxidative stress markers.

Oxidative stress markers

The Malondialdehyde (MDA) concentrations of brain tissue were assessed using the reaction of Thiobarbituric acid as previously described (21). Catalase (CAT) activity in homogeneous brain tissue was evaluated based on its ability to decompose H_2O_2 using the Aebi method (22). The amount of total antioxidant capacity (TAOC) of brain tissue was evaluated using the ferric reducing antioxidant power (FRAP) test (23).

Biochemical analysis

Serum total cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were determined using the diagnostic kit (Pars Azmoon Kit, IRI) on an automatic analyzer (Abbott, model Alcyon 300, USA).

Statistical Analysis

Statistical analysis was performed by SPSS software (version 24.0, IBM Corp., Armonk, USA) using a one-way ANOVA and Tukey's post hoc tests. A p-value of less than 0.05 was considered statistically significant.

Results

Working memory outcome

The working memory rate in the SimH group was significantly increased compared to the controls ($p<0.05$). While the SimL and Vit D groups did not show a significant difference from the control group ($p>0.05$). However, the working memory rate in the SimL + Vit D and SimH + Vit D groups was significantly increased compared to the control ($p < 0.05$; Chart 1).

Anxiety results

Statistical results of anxiety rate showed that the anxiety rate in the SimH groups was significantly reduced compared to the control group ($p < 0.05$). However, the anxiety rate in the Vit D and SimL groups was not significantly different from the control ($p > 0.05$). The administration of both high and low doses of simvastatin with vitamin D in the SimL + Vit D and SimH + Vit D groups significantly reduced the anxiety rate in these groups compared to the control group ($p < 0.05$; Chart 1).

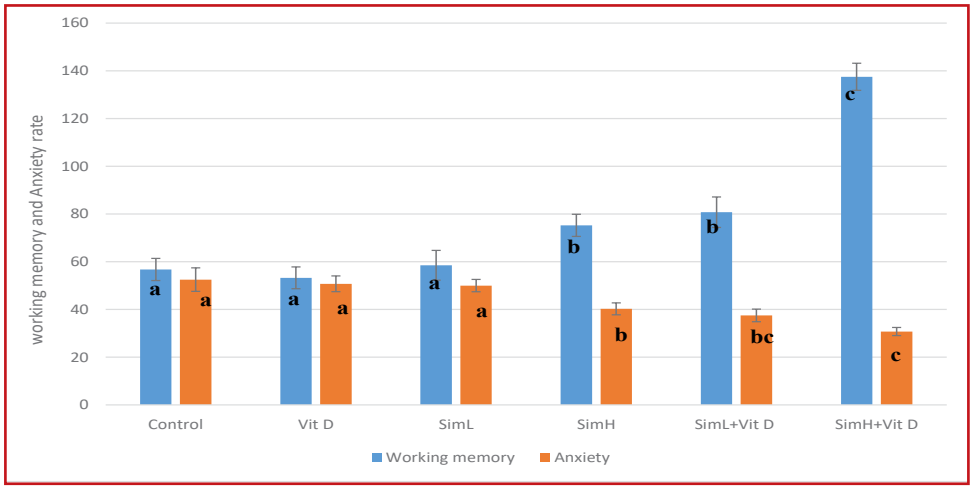


Chart 1. Effect of simvastatin and vitamin D (Vit D) on working memory and Anxiety rate in different groups (Mean ± SEM)

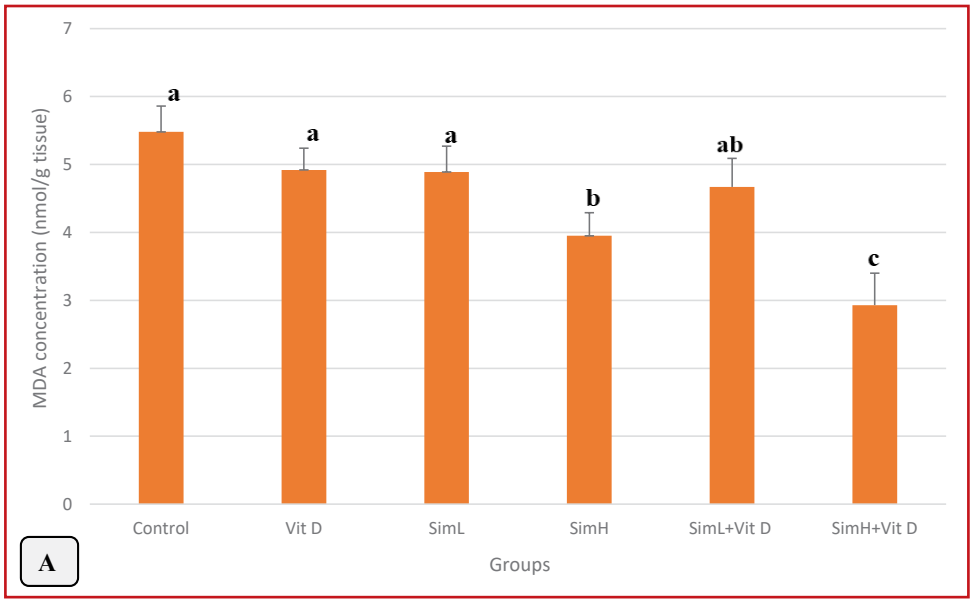
[abc] Different letters indicate a significant difference between groups in each column ($p < 0.05$).

Oxidative stress markers outcome

The MDA concentration in the SimH group showed a significant decrease compared to the control ($p>0.05$). However, no significant change was observed in the Vit D and SimL groups compared to the control group ($p<0.05$). Co-administration of simvastatin and vitamin D in the SimH + Vit D group significantly reduced MDA compared to the control group ($p>0.05$; Chart 2-A).

Catalase activity in SimH, SimL + Vit D, and SimH + Vit D groups significantly increased compared to the control group ($p<0.05$). However, the increase in catalase activity in the SimL and Vit D groups was not statistically significant with the control group ($p>0.05$; Chart 2-B).

The level of TAOC in SimH, SimL + Vit D, and SimH + Vit D groups were significantly increased compared to the control group ($p<0.05$). However, the increase in TAOC levels in Vit D and SimL groups compared to the control group was not statistically significant ($p > 0.05$; Chart 2-C).



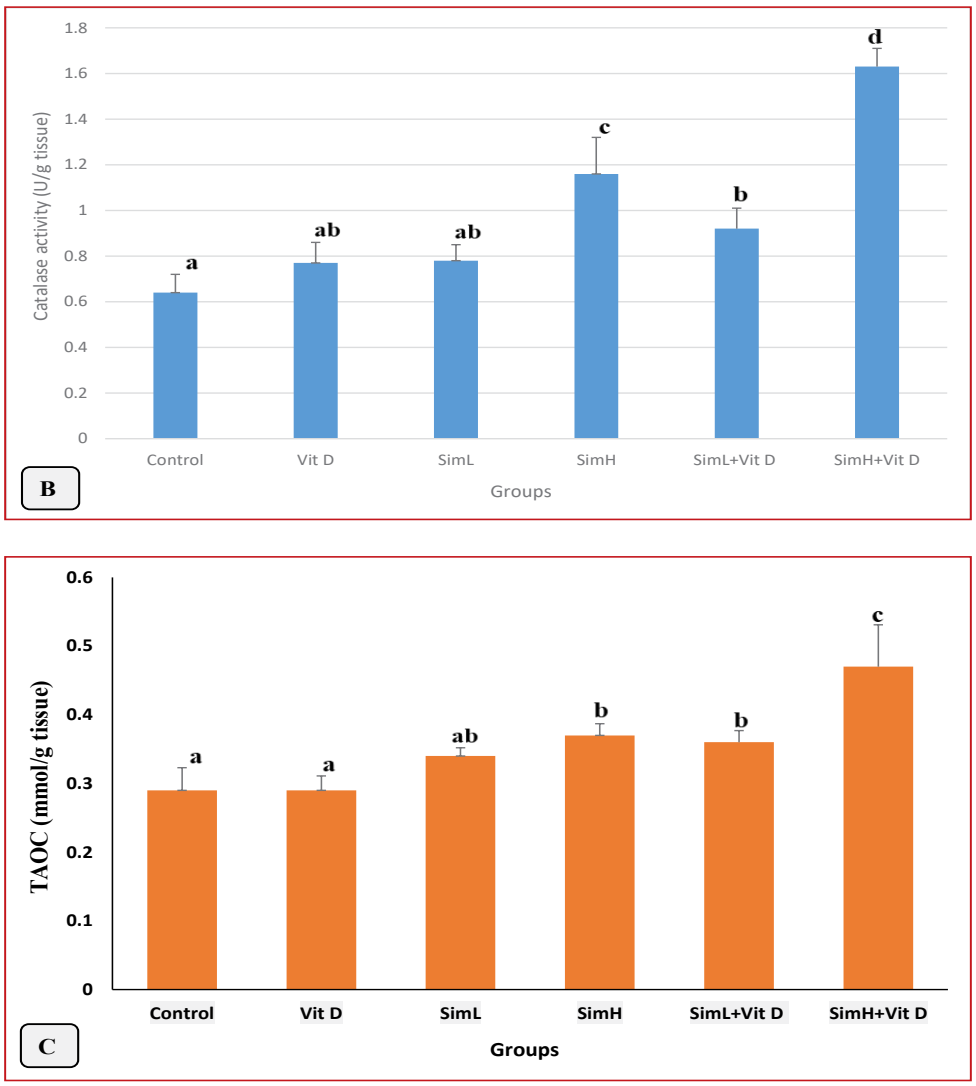


Chart 2. Effect of simvastatin and vitamin D (Vit D) on malondialdehyde (MDA) concentration, Catalase activity, and total antioxidant capacity (TAOC) in different groups (Mean ± SEM)

[abcd] Different letters indicate a significant difference between groups in each column ($p < 0.05$)

Serological outcome

The serum total cholesterol levels in the SimH group showed a significant increase compared to the control group ($p < 0.05$). While the level of total cholesterol in the SimL and Vit D groups did not show a significant difference compared to the control group ($p > 0.05$). There was a significant increase in the SimH + Vit D group compared to the control group ($p < 0.05$). However, the increase in total cholesterol level in the SimL + Vit D group compared to the control group was not statistically significant ($p > 0.05$; Table 1).

The LDL-C levels were significantly increased in the SimH group compared to the control group ($p < 0.05$). While LDL-C in SimL and Vit D groups were not significantly different from control ($p > 0.05$). However, there was a significant increase in the SimL + Vit D and SimH + Vit D groups compared to the control group ($p < 0.05$; Table 1).

The HDL-C levels in SimH and SimH + Vit D groups showed a significant increase compared to the control group ($p > 0.05$). However, the increase of HDL-C levels in Vit D, SimL, and SimL + Vit D groups was not statistically significant compared to the control group ($p < 0.05$; Table 1).

Table 1. Effect of simvastatin and vitamin D (Vit D) on total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density cholesterol (HDL-C) in different groups (Mean ± SEM)

Groups	Total cholesterol (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Control	83.89 ± 4.66 ^{ab}	28.11 ± 1.60 ^{ab}	30.23 ± 2.05 ^{a}
Vit D	85.95 ± 3.60 ^{a}	29.15 ± 1.56 ^{a}	30.91 ± 2.47 ^{a}
SimL	77.25 ± 4.40 ^{b}	25.76 ± 1.84 ^{ab}	31.16 ± 3.44 ^{a}
SimH	57.67 ± 2.35 ^{c}	12.43 ± 2.21 ^{c}	48.27 ± 2.31 ^{b}
SimL + Vit D	80.44 ± 3.78 ^{ab}	25.03 ± 1.96 ^{b}	33.69 ± 2.59 ^{a}
SimH + Vit D	59.60 ± 2.48 ^{c}	14.71 ± 1.29 ^{c}	49.28 ± 2.37 ^{b}

^{abc} Different letters indicate a significant difference between groups in each column ($p < 0.05$)

Discussion

One of the highest functional levels of the central nervous system is learning and memory. Learning is a neural phenomenon in which an organism changes its behavior through practice, while memory refers to the process of storing what is learned (24). Studies have shown that short-term memory is associated with the cerebral cortex and long-term memory is associated with the limbic apparatus, however, no specific location for memory storage has been identified because memory is not lost altogether by removing different parts of the brain (25).

The present study showed that rats receiving simvastatin at a dose of 10 mg/kg as well as simvastatin plus vitamin D had significantly increased working memory compared with controls. This result contradicts the conclusions of Baytan et al., regarding the adverse effects of simvastatin at a dose of 10 mg/kg. The results of their psychomotor function and spatial memory tests indicate that long-term simvastatin usage impairs spatial memory only at 10 mg/kg/day dose (19). However, the results of the present study in this field confirm the research of Douma et al., their results indicate an increase in cognition in healthy rats treated with simvastatin (11). There are many reports of the effects of statins on cognitive function in rodents (26, 27). Some of these studies have reported adverse effects of statins (9, 28, 29), and others have reported positive effects of statins on memory and cognition (12, 11, 30). Researchers have also studied the effects of simvastatin on memory and cognition. One of these studies is the research of Douma et al., their results indicate an increase in cognition in healthy mice treated with simvastatin (11). In this regard, the results of the study by Baytan et al., to investigate the effect of simvastatin on memory, showed contradictory results, so that the use of simvastatin for four weeks at a dose of 10 mg/kg/day caused memory impairment in Barnes maze test. However, this effect was not surprisingly observed in rats that took simvastatin at a dose of 30 mg/kg/day. In other words, their test results showed that long-term use of simvastatin impairs spatial memory at a dose of only 10 mg/kg/day (19). Vitamin D deficiency has recently been shown to be associated with inferior working memory and executive function; and vitamin D can improve memory and cognition (31).

Studies on the anxiety level have shown that chronic exposure to simvastatin reduced anxiety levels in Mozart-stimulated rats (32). The results of studies by Yang et al., also confirm the positive effect of simvastatin on reducing anxiety (33).

Although preclinical studies provide limited evidence on the possible mechanisms underlying the beneficial effects of vitamin D for the management of these disorders, most of the clinical studies have indicated that vitamin D supplementation is associated with the reduction of symptoms of depression and anxiety (34). The present study confirms the results of the mentioned research on the effect of statins and vitamin D on reducing anxiety.

There is no direct correlation between brain cholesterol level, as well as HMG-CoA activity with memory function regulation. However, there is a definite link between plasma cholesterol level and brain acetylcholine esterase activity level. A long-standing plasma cholesterol alteration may be essential to regulate memory function which in turn might be mediated through the brain acetylcholine esterase modulated pathway (35). Previous studies have shown the beneficial effects of vitamin D supplementation on glucose homeostasis and HDL cholesterol levels (36). All statins are competitively attached to the catalytic region of HMG-COA reductase, creating an ester barrier to HMG-CoA access to the active site of the LDL-C receptor, which enhances LDL-C reuptake and LDL-C precursors from Systemic circulation causes proteins that bind to the sterol regulatory unit to sense changes in cholesterol levels and subsequently increase the expression of LDL-C receptors to reabsorb LDL-C from serum, to compensate for cellular cholesterol depletion. As a result, a significant proportion of cholesterol-lowering statins indirectly lead to the clearance of LDL-C from plasma (37).

The results of the present study showed a significant increase in the level of Catalase and TAOC and a significant decrease in the MDA level of brain tissue in the SimH and SimH + Vit D groups. Statins exhibit pleiotropic effects such as decreasing inflammation, oxidative stress, and reducing tumor progression (38). The molecular basis of the anti-inflammatory effects observed from statins may be related to their ability to stop the production or activity of ROS. The antioxidant effects of statins are likely to contribute to their clinical effectiveness in the treatment of cardiovascular disease as well as other chronic diseases associated with increased oxidative stress in humans (39). Studies have shown that a decrease in vitamin D levels in the brain is associated with an increase in MDA and a decrease in GP and SOD, indicating the antioxidant properties of vitamin D in the brain (40). Malondialdehyde is the end product of fat peroxidation and is widely used as a marker of oxidative stress (41). Significant reductions in serum MDA as a marker of lipid peroxidation have been reported in patients treated with high-dose simvastatin for at least six months (42). TAOC levels take into account the cumulative action of all antioxidants present in plasma and body fluids and provide an integrated parameter rather than a simple set of measurable antioxidants (43). Research results show that TAOC levels increase in patients treated with atorvastatin (44). Simvastatin increases catalase levels and inhibits lipid peroxidation (45). The results of this part of the present study in line with the mentioned studies confirm the positive antioxidant effects of simvastatin and vitamin D.

The strong point of this study is the simultaneous evaluation of behavioral tests and oxidative stress markers in the brain with blood cholesterol levels, which shows a clear view of the relationship between cholesterol levels and behavior. The limitations of the present study include the short duration of treatment, the lack of histological examination of the hippocampus, and genes associated with apoptosis such as Caspases and Bcl-2. Therefore, to obtain better results, a longer duration of treatment, apoptosis genes expression, and histological examination of the hippocampus should be selected in future studies.

Conclusion

This study demonstrated that the dose-dependent simvastatin could increase working memory and reduce anxiety levels in rats in the presence of vitamin D by reducing oxidative stress and cholesterol in the serum profile. However, more research is needed to evaluate the effect of this drug on other parameters and organs.

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Conflict of Interests

All the authors express that there is no conflict of interest regarding the publication of this article.

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