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Research Article

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Effect of Simvastatin Intracerebroventricular Injection (ICV) On Memory and Anxiety in Male Rats in The Presence of Vitamin D Supplementation

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Abstract

Introduction: *Simvastatin is a lipophilic statin. Lipophilic statins can cross the blood-brain barrier. The present study investigates the effect of simvastatin on working memory and anxiety in adult male rats without brain injury by the ICV method.*

Method: *In this experimental study, 26 male Wistar rats weighing 250-300 g were randomly divided into six groups (n=6) including: control ICV (without injection), Shm (3µl ICV distilled water), simvastatin 28.5 nmol ICV, simvastatin 28.5 nmol ICV + vitamin D 5 µg/kg. Simvastatin treatment was performed for seven days. After the treatment period, the working memory and anxiety was measured.*

Findings: *According to the results, groups receiving simvastatin with a concentration of 28.5 nmol had no significant difference in their memory and anxiety than the control group (P> 0.05). No significant effect on catalase level was observed in any of the groups compared to the control group (P> 0.05).*

Conclusion: *Injection of simvastatin into the brain did not show a significant effect on memory and anxiety in male rats.*

Keywords: Anxiety, Catalase, ICV, Simvastatin, Vitamin D, Working Memory.

Introduction

Inhibitors of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, statins are approved by the US Food and Drug Administration (FDA) as cholesterol-lowering drugs. These drugs target the liver and inhibit cholesterol biosynthesis, which results in decreased intracellular cholesterol levels in the liver and subsequently regulates the number of LDL-C receptors on the cell surface [1]. Statins are generally known for protecting and treating cardiovascular disease [2], reducing LDL-C and TG levels, and increasing HDL-C [3, 4]. Lipid-lowering drugs, such as statins, are the most commonly applicable drugs. In fact, between 2007 and 2011, only over 2.9 million people in Canada consumed lipid-lowering drugs [5]. Research on animals and humans has shown that simvastatin is a lipophilic statin capable of crossing the blood-brain barrier [6, 7], so it simply affects the brain. Depending on their lipophilicity rank, different types of statins cross the blood-brain barrier with different efficiencies [7, 8]. Lipophilic statins such as simvastatin in the nervous system accelerate cerebral healing trauma, improve learning in mice, increase protein synthesis and differentiation of cells into adult neurons in mice and increase the expression of Brain-derived neurotrophic factor (BDNF) and Vascular endothelial growth factor (VEGF) [9].

Oxidative stress stems from the imbalance relation of oxidants (free radicals) and antioxidants, which results in the accumulation of free radicals in the cells, which subsequently interact with the biological molecules. It mainly arises from an increase in free radicals, an imbalance between the production and elimination of active species in the body, and a decrease in the potency of the antioxidant defense system [10, 11]. Several cellular processes, including cell metabolism, cell signaling pathways, gene expression, cell proliferation, and programmed cell death, are affected by oxidative stress [12]. Antioxidants are the primary protective mechanism against oxidants, and catalase is the most known antioxidant which plays an important role in removing hydrogen peroxide. It enzymatically converts hydrogen peroxide into water and oxygen molecules [13].

Statins have rapid anti-inflammatory and antioxidant effects [14]. It has also been reported that statins regulate antioxidant enzymes such as catalase [15, 16]. Simvastatin has been shown to increase tissue catalase [17-19]. In this regard, it has also been reported that overexpression of catalase may enhance cognition [20].

There is a wide deal of research about the effects of statins on memory. In the past decade, statins have been reported in some cases to increase the risk of cognitive impairments, such as memory loss and amnesia [21, 22]. In February 2012, the US Food and Drug Administration(FDA) [23], announced that "memory loss" and "confusion" were among the reported cognitive effects in patients treated with statins [24-26]. However, some research results indicate the positive effects of statins on cognitive and memory performance in laboratory animals and humans [27, 28]. In the present study due to the discrepancy regarding the effects of statins on cognitive performance whether negative [21, 29] or positive effects [28], the direct effects of simvastatin by ICV injections on the memory and anxiety of healthy male rats were investigated.

Materials and methods

Twenty-six male Westar rats weighing 250-300g were purchased from the animal house of Urmia University. All procedures were followed according to the National Institute of Health guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and local guidelines for compassionate use of animals in research; Rats were kept in cages with open access to standard tap water and compact chow. The animals were held in the same laboratory conditions (18°C to 23°C room temperature and controlled humidity) with alternating 12-h light and dark cycles. The Ethics Committee has approved the proposal of this study of Urmia University (Ethics Code: IR-UU-AEC-3/1033 / DA).

Animal grouping

1. Healthy control group who received normal water were not injected (C).
2. Sham group received 3 ul of distilled water as ICV (shm)
3. Simvastatin group with a dose of 28.5 nmol received a simvastatin by ICV[30] (sim).
4. Simvastatin group with a dose of 28.5 nmol by ICV with vitamin D supplement (Vitamin D was injected intraperitoneally) 5 µg/kg (200 IU) [31] (sim + vD).

The drugs were administered once daily for seven days. The drug simvastatin used in the present study was produced by Timova Pharmaceutical Company (Denmark).

Stereotaxic surgery and cannulation for intraventricular brain group

A cannula was implanted in the third cerebral ventricle of the rats a week before the onset of behavioral tests. Before surgery, animals were anesthetized by intraperitoneal injection of a combination of ketamine and xylazine at doses of 10 mg/kg and 10 mg/kg, respectively [32] . After shaving head and disinfecting hair with 10% betadine and 70% alcohol, they were placed in stereotaxic apparatus, then using third ventricular coordinate axes (AP = -4.2 mm, L = 0mm, DV = -4.2), a 5mm long cannula (needle head 23) was placed as a guiding cannula in the third ventricle of their brain. The coordinate axes used in stereotaxis to access the brain's third ventricle were selected from the brain atlas [33] to fix the cannula on the animal skull using two eyeglass screws as a base dental cement (a mixture of self-curing acrylic and monomer). A piece of thin copper wire was inserted into the cannula to prevent CSF or blood flow through the guiding cannula.

Memory and anxiety tests were performed at the end of the treatment period.

Measurement Of The Working Memory In The Cross-Maze

cross-maze is made of wood and has four equal arms measured 13*50*10 1cm 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. Entering the animal into one arm was when the animal's hind legs were fully inserted into the arm. Periodic behavior was considered 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 spins observed to the number of possible spins (Total number of arms entering - 3) 100 *.

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

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

Measurement of The Anxiety in The Plus-Maze

Elevated plus maze (EMP) is used to measure anxiety[34] . This assessment is based on two instincts: the sense of rodent search and 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 with four arms in the form of a plus-Maze. During the 5 minutes 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, how long the animal stayed in the open arm, how long the animal remains in the closed arm (The entry of the open or closed arm means that all four legs of the animal are in the target arm, the length of stay in each arm was calculated accordingly).

In the present study, a significant increase in the percentage of time spent in the open arm indicates a decrease in anxiety in rats.

Percentage of time spent in the open arm

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

Measurement Of Brain Catalase Levels

Aebi method was used to measure catalase activity [35]. To a cer-

tain volume of tissue extract, absolute ethanol (0.01 ml) was added and incubated in ice for 34 minutes. Then Triton 14% X-100 was added at a final concentration of 1%. This solution was used to measure enzyme activity. The reaction was initiated by adding 0.05 ml of 30 mM H_2O_2 L to the tissue sample in 50 mM potassium phosphate buffer at pH = 7. The adsorption was then read at 240 nm for 3 min (a unit of catalase activity was 1 µmol of H_2O_2 decomposing in a minute). Enzyme activity was calculated in units of mg protein. A transverse incision was made from the cortex to the base of the brain to obtain the desired tissue sample size.

Statistical Analysis

Data were analyzed by one-way ANOVA using SPSS 19 software and Tukey tests, and the results were presented as mean ± standard deviation error. P <0.05 was considered as the level of significance.

Results

Working Memory Test Results (In Cross-Maze)

Statistical results of behavioral memory test (based on rodent curiosity in exploring the environment and avoiding repetitive arms that determine the number of true rotations) on mice showed that none of the groups had a significant difference in working memory compared to the control group (P> 0.05) (Figure 1).

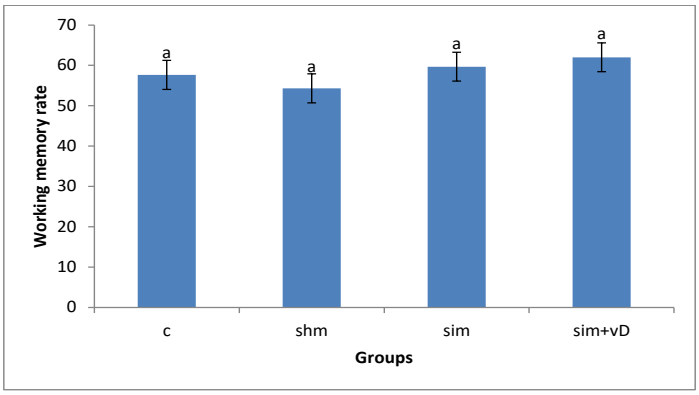

Figure 1: Effect of simvastatin and vitamin D (VD) on working memory rate in different groups (Mean ± SEM). abc Different letters indicate a significant difference between groups in each column ($p < 0.05$).

Anxiety Test Results (Plus-Maze)

The statistical results of the Behavioral Anxiety Test on mice showed that none of the groups were significantly different from the control group (P> 0.05) (Figure 2).

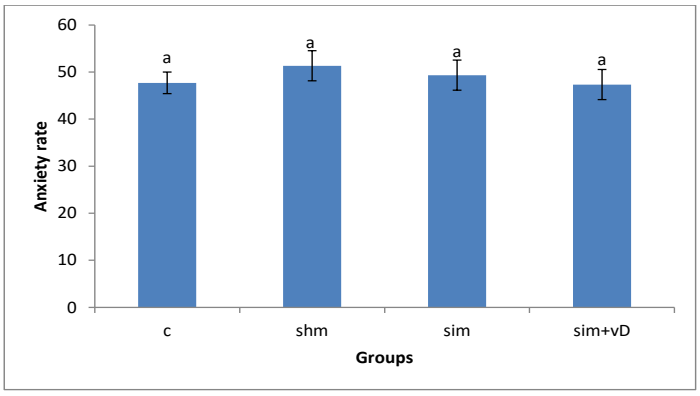

Figure 2: Effect of simvastatin and vitamin D (VD) on anxiety rate in different groups (Mean ± SEM). abc Different letters indicate a significant difference between groups in each column ($p < 0.05$).

Catalase biochemical test results.

Statistical results of brain tissue catalase level showed that the groups receiving ICV simvastatin or distilled water did not have a significant difference in brain tissue catalase (P> 0.05) (Figure 3).

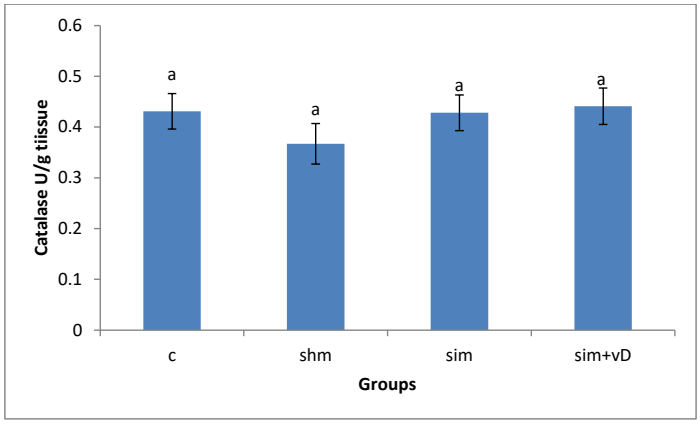

Figure 3: Effect of simvastatin and vitamin D (VD) on Catalase activity in different groups (Mean ± SEM). abc Different letters indicate a significant difference between groups in each column ($p < 0.05$).

Discussion and conclusion

Several studies have investigated the effects of statins on memory in laboratory animals and humans [28, 36], however, limited research has been conducted to investigate the impact of statins on the memory of healthy animals [28]. There is some limitations study along with the present study to investigate the injection of ICV statins into the cerebral ventricle (In the present study, the injection of ICV was performed to investigate the direct effect of simvastatin on the brain and its secondary effects on memory) [30]. This was the reason for the necessity of this research.

Learning and memory are one of the highest levels of functioning of the central nervous system. Learning is a neurotic phenomenon in which animal changes their behavior through practice, while memory refers to the process of storing the learned [37]. Learning and memory involve a wide range of changes in the structure

and function of the nervous system, mainly confined to synapses involved in the direction of the message and sensory information in the nervous system. The structural change involves several synapses and changes in the extent of postsynaptic membranes at the site of contact, and physiological changes include those in the ionic conductivity of the pre-and postsynaptic membranes [38]. Short-term memory has been shown to correlate with cortex and long-term memory with the limbic tract. Still, no specific storage location has been identified so far because removal of different parts of the brain generally does not eliminate memory [39].

The hippocampus is part of the limbic tract. The limbic tract is involved in the long-term memory process. The hippocampus receives inputs from the prefrontal cortex and demonstrates the role of the prefrontal cortex in the short-term memory process [39], in addition, different areas of the brain play a role in memory and cognition, including the Nuclear Regulatory Miner (NBM), which is equivalent to the rodent magnocellular nucleus, which is responsible for many cognitive and memory deficits [40]. About 90% of NBM nucleus neurons are cholinergic and transmit their fibers to all amygdala cortical and nuclear regions [41]. The NMB nucleus is a primary source of cholinergic divisions to the hippocampus and cortex [42]. The cholinergic system is responsible for storing and retrieving memory information, and most neurons in the NMB are cholinergic [43]. Studies show that oral administration of lipophilic statins reduces acetylcholinesterase activity in the frontal cortex of mice, which leads to increased levels of acetylcholine in the synaptic cleft and reduces cholinergic dysfunction even in mice. It becomes Alzheimer's disease. The lipophilic drug, such as lovastatin, clearly improves the activity of muscarinic and NMDA receptors in the cortex and hippocampus. Increased function of these receptors in the prefrontal cortex results in enhanced memory, resulting in lipophilic statins treatment [44-46]. Lipophilic statins enzyme activity increases choline acetyltransferase in the frontal cortex and hippocampus [47]. The acetylcholine system plays an important role in memory and recovery; the hippocampal areas of the amygdala and the cortex are important target sites for acetylcholine neurotransmitters, which play an important role in-memory processing. [48, 49]. Memory and learning are among the most evolved functions of the nervous system. Various experiences have shown that the activity of the cholinergic systems of the brain plays a major role in the memory and learning process [50, 51].

There are numerous reports of the effects of statins on cognitive function in rodents [36, 52]. Some of these studies have reported the adverse effects of statins [21, 29], and others have noted the positive impact on memory and cognition [28]. Researchers have also examined the effects of simvastatin on memory and cognition, one of which is the study by Duma et al., which results in increased cognition in healthy mice treated with simvastatin [28]. In line with the results of the study by Bitan et al. to investigate the effect of simvastatin on memory, showed contradictory results, using simvastatin for four weeks at doses 10 mg/kg. However, surprisingly, this effect was not observed in the mice receiving simvastatin at a 30 mg/kg dose. The results were not significant. Their tests showed that long-term use of simvastatin impaired spatial memory at a dose of only 10 mg/kg per day [53]. However, in the present study, injection of simvastatin into the third ventricle of the brain as ICV at a dose of 28.5 nmol did not significantly affect working memory in rats (Figure 1). A study by Ranaiy et al. In 2021 shows an increase in working memory of male rats treated with simvastatin 10 mg [54]. However, no significant effect of simvastatin was observed in the present study on working memory. The low dose of the drug is probably the reason for the ineffectiveness of simvastatin on working memory in the present study. the results of Ramirez et al. (Ramirez, et al., 2011) showed that amongst the statins tested, simvastatin only enhanced episodic-like memory. Their results suggest that simvastatin and lovastatin (especially simvastatin) may have good therapeutic potential in the treatment of neurodegenerative disorders and memory impairment [55]. The results of some research on laboratory animals and humans also indicate that in the long term, simvastatin treatment decreases memory [53, 56]; however, the present study results do not show any adverse effects of simvastatin.

Statins have rapid antioxidant effects [14]. tatins have been reported to regulate antioxidant enzymes such as catalase [15, 16]. Simvastatin, a lipophilic member of the statins family, has antioxidant properties [57]. Increased tissue catalase levels have been reported as one of the antioxidant effects of simvastatin [17, 58]. Over-expression of catalase may enhance cognition [20]. Decreased catalase activity is associated with hippocampal-dependent spatial memory impairment [59]. Studies show that lowering hydrogen peroxide levels is beneficial to cognitive health [60]. In the study of Olson et al. (Olsen, R.H., et al), the activity of catalase was assessed in all brain regions in mice and an increase in the level of catalase was observed in all brain regions. Olson et al. reported that cognitive performance improvement was due to the effect of catalase on endogenous H2O2 and subsequent improvement in physiological processes such as synaptic flexibility and LTP in mice, which supports the important role of catalase in improving cognitive function [20]. The results of injection of simvastatin ICV and in the rats in the present study did not show a significant difference in the increase of catalase compared to the control group which this result can be due to the short duration of the treatment period (injection into the ventricle of the brain for seven days) and the daily injection rate (28.5nmol). However, in the present study, the level of brain tissue catalase under the influence of simvastatin did not show any significant change compared to the control group (Figure 3). In the present study, molecular processes and factors that may affect the effect of catalase levels on neurons and memory were not evaluated, and due to the clinical importance of simvastatin, this part of the present study needs further research in the future. It should be noted that the present study investigated the direct effect of simvastatin on the brain of healthy male rats, but there is very little research similar to this study [30].

Data collected over the past decade suggest that NMDA receptors may play a role in the pathophysiology of depression and its mechanism of action [61]. In addition, there is evidence of the therapeutic effects of simvastatin on NMDA receptor binding density

in the brain, which acts as a potential factor in reducing anxiety [62]. The study results of Tani Synthesis and Mona Lisa Marizati Bangraz et al. The behavioral interactions of simvastatin and fluoxetine in the anxiety and depression test also showed a decrease in anxiety associated with the use of simvastatin [63]. A study suggests that chronic exposure to simvastatin reduces anxiety levels in Mozart music-stimulated rats [64]. The results of studies by Yingong Young-XU et al. confirm the positive effect of simvastatin on reducing anxiety [65].

However, the results of this part of the present study, despite the difference in the type of simvastatin (in the present study as an intraventricular injection) and the difference in the dose used in previous studies (in the present study, the dose of 28.5 nmol) and the test device (Plus-Maze), indicating that simvastatin did not affect reducing or increasing rat anxiety. In other words, the present study results did not show any significant differences in the anxiety of the experimental groups compared to the control group. Also, in this study, the direct effect of simvastatin on the anxiety of healthy mice was investigated. It should be noted that the presence of vitamin D supplementation has a significant impact on reducing or increasing anxiety in the receiving groups did not show it compared to the control group (Figure 2). The lack of effect of the applied dose of simvastatin in the present study on the level of catalase is also consistent with the lack of significant effect of this dose of the drug on the level of memory and anxiety.
Overall, the present study showed that low-dose simvastatin, even if injected directly into the nervous system, did not significantly affect memory and anxiety. This situation could be key for future researchers to investigate other drug effects on the CNC . It should be noted that the results of the present study need further study in the future.

Conclusion
Injection of simvastatin into the brain did not show a significant effect on memory and anxiety in male rats.

Abbreviations
BDNF: Brain-derived neurotrophic factor
VEGF: Vascular endothelial growth factor
CNC: Central nervous system
HDL-C: High -Density Lipoproteins
HMGCR: Hydroxy Methyl Glutaryl CoA-reductase
KG: Kilogram
LDL-C: Low-Density Lipoproteins
MG: Milligram
NMDA: N-methylD-aspartate
SIM: Simvastatin
VD: Vitamin D
µg: Micrograms

Authors' Contributions
All authors read and approved the final manuscript.

Ethical Approval and Consent to participate
This article is extracted from the dissertation of Mohammad Saleh Ranaiy and all authors are satisfied with their participation. The proposal of this study has been approved by the Ethics Committee of Urmia University (Ethics Code: IR-UU-AEC-3/1033 / DA).

Consent for publication
All authors are fully satisfied with the publication of this article.

Availability of supporting data
There is data to support this article.

Conflict of Interest
There is no authors' conflict of interest

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Authors' contributions
All authors have contributed to this article.

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References

1. Brown, M. S., & Goldstein, J. L. (1999). A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. Proceedings of the National Academy of Sciences, 96(20), 11041-11048.
2. Taylor, F., Ward, K., Moore, T. H., Burke, M., Smith, G. D., Casas, J. P., & Ebrahim, S. (2011). Statins for the primary prevention of cardiovascular disease. Cochrane database of systematic reviews, (1).
3. Karalis, D. G. (2009, April). Intensive lowering of low-density lipoprotein cholesterol levels for primary prevention of coronary artery disease. In Mayo Clinic Proceedings (Vol. 84, No. 4, pp. 345-352). Elsevier.
4. Liao, J. K., & Laufs, U. (2005). Pleiotropic effects of statins. Annu. Rev. Pharmacol. Toxicol., 45, 89-118.
5. Rotermann, M., Sanmartin, C., Hennessy, D., & Arthur, M. (2014). Prescription medication use by Canadians aged 6 to 79. Health reports, 25(6), 3.
6. Botti, R. E., Triscari, J., Pan, H. Y., & Zayat, J. (1991). Concentrations of pravastatin and lovastatin in cerebrospinal fluid in healthy subjects. Clinical neuropharmacology, 14(3), 256-261.
7. Saheki, A., Terasaki, T., Tamai, I., & Tsuji, A. (1994). In vivo and in vitro blood–brain barrier transport of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. Pharmaceutical research, 11(2), 305-311.
8. Tsuji, A., Saheki, A., Tamai, I., & Terasaki, T. (1993). Transport mechanism of 3-hydroxy-3-methylglutaryl coenzyme

A reductase inhibitors at the blood-brain barrier. Journal of Pharmacology and Experimental Therapeutics, 267(3), 1085-1090.
9. Wu, H., Lu, D., Jiang, H., Xiong, Y., Qu, C., Li, B., ... & Chopp, M. (2008). Simvastatin-mediated upregulation of VEGF and BDNF, activation of the PI3K/Akt pathway, and increase of neurogenesis are associated with therapeutic improvement after traumatic brain injury. Journal of neurotrauma, 25(2), 130-139.
10. Dayem, A. A., Choi, H. Y., Kim, J. H., & Cho, S. G. (2010). Role of oxidative stress in stem, cancer, and cancer stem cells. Cancers, 2(2), 859-884.
11. Tandon, V. R., Sharma, S., Mahajan, A., & Bardi, G. H. (2005). Oxidative stress: a novel strategy in cancer treatment. Jk Science, 7(1), 1-3.
12. Poli, G., Leonarduzzi, G., Biasi, F., & Chiarpotto, E. (2004). Oxidative stress and cell signalling. Current medicinal chemistry, 11(9), 1163-1182.
13. Turrens, J. F., Crapo, J. D., & Freeman, B. (1984). Protection against oxygen toxicity by intravenous injection of liposome-entrapped catalase and superoxide dismutase. The Journal of clinical investigation, 73(1), 87-95.
14. Pinho-Gomes, A. C., Reilly, S., Brandes, R. P., & Casadei, B. (2014). Targeting inflammation and oxidative stress in atrial fibrillation: role of 3-hydroxy-3-methylglutaryl-coenzyme a reductase inhibition with statins. Antioxidants & redox signaling, 20(8), 1268-1285.
15. Wassmann, S., Laufs, U., Müller, K., Konkol, C., Ahlbory, K., Bäumer, A. T., ... & Nickenig, G. (2002). Cellular antioxidant effects of atorvastatin in vitro and in vivo. Arteriosclerosis, thrombosis, and vascular biology, 22(2), 300-305.
16. Aviram, M., Hussein, O., Rosenblat, M., Schlezinger, S., Hayek, T., & Keidar, S. (1998). Interactions of platelets, macrophages, and lipoproteins in hypercholesterolemia: antiatherogenic effects of HMG-CoA reductase inhibitor therapy. Journal of cardiovascular pharmacology, 31(1), 39-45.
17. Guterres, F. A. D. L. B., Martinez, G. R., Rocha, M. E. M., & Winnischofer, S. M. B. (2013). Simvastatin rises reactive oxygen species levels and induces senescence in human melanoma cells by activation of p53/p21 pathway. Experimental cell research, 319(19), 2977-2988.
18. Mohamadin, A. M., Elberry, A. A., Abdel Gawad, H. S., Morsy, G. M., & Al-Abbasi, F. A. (2011). Protective effects of simvastatin, a lipid lowering agent, against oxidative damage in experimental diabetic rats. Journal of lipids, 2011.
19. Eger, G. A., Ferreira, V. V., Batista, C. R., Bonde, H., Lima, D. D. D., Wyse, A. T., ... & da Cruz, J. G. (2016). Antioxidant effect of simvastatin throught oxidative imbalance caused by lisdexamfetamine dimesylate. Anais da Academia Brasileira de Ciências, 88, 335-348.
20. Olsen, R. H., Johnson, L. A., Zuloaga, D. G., Limoli, C. L., & Raber, J. (2013). Enhanced hippocampus-dependent memory and reduced anxiety in mice over-expressing human catalase in mitochondria. Journal of neurochemistry, 125(2), 303-313.
21. Jacobson, T. A. (2014). NLA task force on statin safety-2014 update. Journal of clinical lipidology, 8(3), S1-S4.
22. Bayat, M., Baluchnejadmojarad, T., Roghani, M., Goshadrou, F., Ronaghi, A., & Mehdizadeh, M. (2012). Netrin-1 improves spatial memory and synaptic plasticity impairment following global ischemia in the rat. Brain research, 1452, 185-194.
23. Swiger, K. J., Manalac, R. J., Blumenthal, R. S., Blaha, M. J., & Martin, S. S. (2013, November). Statins and cognition: a systematic review and meta-analysis of short-and long-term cognitive effects. In Mayo clinic proceedings (Vol. 88, No. 11, pp. 1213-1221). Elsevier.
24. Benito-León, J., Louis, E. D., Vega, S., & Bermejo-Pareja, F. (2010). Statins and cognitive functioning in the elderly: a population-based study. Journal of Alzheimer's Disease, 21(1), 95-102.
25. Bettermann, K., Arnold, A. M., Williamson, J., Rapp, S., Sink, K., Toole, J. F., ... & Burke, G. L. (2012). Statins, risk of dementia, and cognitive function: secondary analysis of the ginkgo evaluation of memory study. Journal of Stroke and Cerebrovascular Diseases, 21(6), 436-444.
26. Evans, M. A., & Golomb, B. A. (2009). Statin-associated adverse cognitive effects: survey results from 171 patients. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 29(7), 800-811.
27. Sparks, D. L., Kryscio, R. J., Connor, D. J., Sabbagh, M. N., Sparks, L. M., Lin, Y., & Liebsack, C. (2010). Cholesterol and cognitive performance in normal controls and the influence of elective statin use after conversion to mild cognitive impairment: results in a clinical trial cohort. Neurodegenerative Diseases, 7(1-3), 183-186.
28. Douma, T. N., Borre, Y., Hendriksen, H., Olivier, B., & Oosting, R. S. (2011). Simvastatin improves learning and memory in control but not in olfactory bulbectomized rats. Psychopharmacology, 216(4), 537-544.
29. Elias, P. K., Elias, M. F., D'agostino, R. B., Sullivan, L. M., & Wolf, P. A. (2005). Serum cholesterol and cognitive performance in the Framingham Heart Study. Psychosomatic medicine, 67(1), 24-30.
30. Cheng, W. H., Ho, W. Y., Chang, C. F., Lu, P. J., Cheng, P. W., Yeh, T. C., ... & Tseng, C. J. (2013). Simvastatin induces a central hypotensive effect via Ras-mediated signalling to cause eNOS up-regulation. British journal of pharmacology, 170(4), 847-858.
31. Dawson-Hughes, B., Harris, S. S., Krall, E. A., & Dallal, G. E. (1997). Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. New England Journal of Medicine, 337(10), 670-676.
32. Moosavi, M., Naghdi, N., Maghsoudi, N., & Asl, S. Z. (2006). The effect of intrahippocampal insulin microinjection on spatial learning and memory. Hormones and behavior, 50(5), 748-752.
33. Paxinos, G., & Watson, C. (2005). The rat brain in stereotaxic coordinates. Burlington, MA.
34. Goldstein, L. E., Rasmusson, A. M., Bunney, B. S., & Roth, R. H. (1996). Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. Journal of Neuroscience, 16(15), 4787-4798.
35. Aebi, H. (1984). [13] Catalase in vitro. In Methods in enzymology (Vol. 105, pp. 121-126). Academic press.
36. Li, L., Cao, D., Kim, H., Lester, R., & Fukuchi, K. I. (2006). Simvastatin enhances learning and memory independent of amyloid load in mice. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society, 60(6), 729-739.
37. Olds, J. A. M. E. S., Disterhoft, J. F., Segal, M. E. N. A. H. E. M., Kornblith, C. L., & Hirsh, R. I. C. H. A. R. D. (1972). Learning centers of rat brain mapped by measuring latencies of conditioned unit responses. Journal of Neurophysiology, 35(2), 202-219.

38. Bracciali, A., Brunelli, M., Cataldo, E., & Degano, P. (2008). Stochastic models for the in silico simulation of synaptic processes. BMC bioinformatics, 9(4), 1-14.
39. Blaise, J. H., Koranda, J. L., Chow, U., Haines, K. E., & Dorward, E. C. (2008). Neonatal isolation stress alters bidirectional long-term synaptic plasticity in amygdalo-hippocampal synapses in freely behaving adult rats. Brain research, 1193, 25-33.
40. Whitehouse, P. J., Price, D. L., Struble, R. G., Clark, A. W., Coyle, J. T., & DeLong, M. R. (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. Science, 215(4537), 1237-1239.
41. Rabiei, Z., Rafieian-Kopaei, M., Heidarian, E., Saghaei, E., & Mokhtari, S. (2014). Effects of Zizyphus jujube extract on memory and learning impairment induced by bilateral electric lesions of the nucleus Basalis of Meynert in rat. Neurochemical research, 39(2), 353-360.
42. Liu, A. K. L., Chang, R. C. C., Pearce, R. K., & Gentleman, S. M. (2015). Nucleus basalis of Meynert revisited: anatomy, history and differential involvement in Alzheimer's and Parkinson's disease. Acta neuropathologica, 129(4), 527-540.
43. Zahedi, M., Hojjati, M. R., Fathpour, H., Rabiei, Z., Alibabaei, Z., & Basim, A. (2015). Effect of Rheum ribes hydro-alcoholic extract on memory impairments in rat model of Alzheimer's disease. Iranian journal of pharmaceutical research: IJPR, 14(4), 1197.
44. Dolga, A. M. (2008). Neuroprotective signaling mechanisms in the mammalian brain. University Library Groningen] [Host].
45. Caccamo, A., Fisher, A., & LaFerla, F. M. (2009). M1 agonists as a potential disease-modifying therapy for Alzheimer's disease. Current Alzheimer Research, 6(2), 112-117.
46. Yamin, G. (2009). NMDA receptor–dependent signaling pathways that underlie amyloid β-protein disruption of LTP in the hippocampus. Journal of neuroscience research, 87(8), 1729-1736.
47. Zhao, Z., Zhao, S., Xu, N., Yu, C., Guan, S., Liu, X., ... & Jia, W. (2010). Lovastatin improves neurological outcome after nucleus basalis magnocellularis lesion in rats. Neuroscience, 167(3), 954-963.
48. Muir, J. L. (1997). Acetylcholine, aging, and Alzheimer's disease. Pharmacology Biochemistry and Behavior, 56(4), 687-696.
49. Uchida, S., & Kagitani, F. (2018). Effect of basal forebrain stimulation on extracellular acetylcholine release and blood flow in the olfactory bulb. The Journal of Physiological Sciences, 68(4), 415-423.
50. Hasselmo, M. E. (2006). The role of acetylcholine in learning and memory. Current opinion in neurobiology, 16(6), 710-715.
51. Tan, H., Liu, N., Wilson, F. A., & Ma, Y. (2007). ORIGINAL INVESTIGATIONS ON CPP: Effects of scopolamine on morphine-induced conditioned place preference in mice. Addiction biology, 12(3-4), 463-469.
52. Abd, T. T., & Jacobson, T. A. (2011). Statin-induced myopathy: a review and update. Expert opinion on drug safety, 10(3), 373-387.
53. Baytan, S. H., Alkanat, M., Okuyan, M., Ekinci, M., Gedikli, E., Ozeren, M., & Akgun, A. (2008). Simvastatin impairs spatial memory in rats at a specific dose level. The Tohoku journal of experimental medicine, 214(4), 341-349.
54. Saleh, R. M., Farah, F., & Farri, B. B. (2022). The Effect of Simvastatin and Vitamin D Co-Administration on Rats Brain Function and Behavior: A Behavioral and Biochemical Study.
55. Ramirez, C., Tercero, I., Pineda, A., & Burgos, J. S. (2011). Simvastatin is the statin that most efficiently protects against kainate-induced excitotoxicity and memory impairment. Journal of Alzheimer's Disease, 24(1), 161-174.\
56. Padala, K. P., Padala, P. R., & Potter, J. F. (2006). Simvastatin-induced decline in cognition. Annals of Pharmacotherapy, 40(10), 1880-1883.
57. Eger, G. A., Ferreira, V. V., Batista, C. R., Bonde, H., Lima, D. D. D., Wyse, A. T., ... & da Cruz, J. G. (2016). Antioxidant effect of simvastatin throught oxidative imbalance caused by lisdexamfetamine dimesylate. Anais da Academia Brasileira de Ciências, 88, 335-348.
58. Mohamadin, A. M., Elberry, A. A., & Gawad, H. S. A. (2011). Protective effects of simvastatin, an HMG-CoA reductase inhibitor, against oxidative damage in experimental diabetic rats.
59. Wang, W., Li, S., Dong, H. P., Lv, S., & Tang, Y. Y. (2009). Differential impairment of spatial and nonspatial cognition in a mouse model of brain aging. Life sciences, 85(3-4), 127-135.
60. Liao, A. C., Craver, B. M., Tseng, B. P., Tran, K. K., Parihar, V. K., Acharya, M. M., & Limoli, C. L. (2013). Mitochondrial-targeted human catalase affords neuroprotection from proton irradiation. Radiation Research, 180(1), 1-6.
61. Szasz, B. K., Mike, A., Karoly, R., Gerevich, Z., Illes, P., Vizi, E. S., & Kiss, J. P. (2007). Direct inhibitory effect of fluoxetine on N-methyl-D-aspartate receptors in the central nervous system. Biological psychiatry, 62(11), 1303-1309.
62. Zhang, X., Tao, Y., Troiani, L., & Markovic-Plese, S. (2011). Simvastatin inhibits IFN regulatory factor 4 expression and Th17 cell differentiation in CD4+ T cells derived from patients with multiple sclerosis. The Journal of Immunology, 187(6), 3431-3437.
63. Santos, T., Baungratz, M. M., Haskel, S. P., de Lima, D. D., da Cruz, J. N., Dal Magro, D. D., & da Cruz, J. G. P. (2012). Behavioral interactions of simvastatin and fluoxetine in tests of anxiety and depression. Neuropsychiatric Disease and Treatment, 8, 413.
64. da Cruz, J. N., de Lima, D. D., Dal Magro, D. D., & da Cruz, J. G. P. (2011). The power of classic music to reduce anxiety in rats treated with simvastatin. Basic and Clinical Neuroscience, 2(4), 5.
65. Young-Xu, Y., Chan, K. A., Liao, J. K., Ravid, S., & Blatt, C. M. (2003). Long-term statin use and psychological well-being. Journal of the American College of Cardiology, 42(4), 690-697.