

# The effect of vitamin E on neuronal apoptosis in hippocampal dentate gyrus in rabbits fed with high-cholesterol diets

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## Abstract

**Background:** Hypercholesterolemia that can increase stress oxidative has destructive effects on brain functions. Vitamin E is a powerful antioxidant and its effects on decrement of oxidative stress in the diseases such as Alzheimer's and hypercholesterolemia are demonstrated. The aim of this study was evaluation of the effects of vitamin E on the level of neuronal apoptosis in granular layer of dentate gyrus in the rabbits that fed with high-cholesterol diet.

**Materials and Methods:** Male New Zealand white rabbits were divided into the control, the Vitamin E (50 mg/kg; gavage), the high-cholesterol diet (containing 2% cholesterol), and the high-cholesterol diet-vitamin E groups. Serum levels of cholesterol, LDL, and HDL, before and after the regimen for 6 weeks, were measured. Then, the rabbits for immunohistochemical staining (TUNEL Test) and evaluation of neuronal apoptosis in dentate gyrus of hippocampal formation were anesthetized and brains were dissected.

**Results:** Results showed that after the regimens, serum levels of cholesterol, LDL, and HDL in the cholesterol receiving groups were increased significantly ( $P < 0.05$ ). Histological results demonstrated that neuronal apoptosis in the dentate gyrus of the high-cholesterol diet group was increased significantly ( $P < 0.05$ ) comparing to the control group; however, vitamin E decreased apoptosis as there wasn't any significant differences between the high-cholesterol diet-vitamin E and control groups.

**Conclusions:** Present results showed that consumption of high-cholesterol diets through hypercholesterolemia and its complication can induce neuronal death in hippocampus and probable resulting cognition disorders; however, vitamin E has neuroprotective effects and prevents neuronal apoptosis significantly.

**Key Words:** Apoptosis, Cholesterol, dentate gyrus, hippocampus, vitamin E

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## INTRODUCTION

Dangerous and growing consumption of fatty foods not only has resulted in the increase of cardiovascular diseases and diabetes (type 2), but also has influenced the evolution of central nervous system, which may disturb cognitive functions through damaging the generation of new neurons in adults' hippocampus<sup>[1]</sup>

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Fatty food is an obvious risk factor of dementia caused by vascular damages and Alzheimer's disease (the most important subspecies of dementia) and also may reduce cognitive functions in the elderly.<sup>[2-5]</sup> It has been found that sugars and fats in the foods, especially saturated fats, transform into cholesterol in body. Cholesterol is an essential component of cell membrane and a precursor for many hormones.<sup>[6,7]</sup> However, studies have shown high blood cholesterol which is largely dependent on diets<sup>[8,9]</sup> and also its metabolic disorders are associated with many central nervous system diseases including Alzheimer's, Parkinson's disease, stroke, etc.<sup>[10-12]</sup> Recently, hypercholesterolemia has been observed to increase neuronal death.<sup>[13]</sup> Fatty foods induce oxidative stress in neurons<sup>[14]</sup> and cholesterol metabolites with cytotoxic effects play an important role in production of reactive oxygen species (ROS).<sup>[10]</sup> Oxidative stress increases neuronal apoptosis (programmed cell death) in areas of central nervous system of mammals, which have neurogenesis also during puberty-like hippocampus. It has been found that oxidative damages along with uncontrolled production of ROS involve in progressive neuronal death in neurodegenerative diseases like Alzheimer's.<sup>[14,15]</sup>

Vitamin E was first identified in green leaves of vegetables in 1992, and it was found as a potent antioxidant in 1989.<sup>[16]</sup> It is a vital and fat-soluble nutrient, which acts as an antioxidant in human body. As vitamin E is not produced in body, people should consume foods containing it. Vitamin E is composed of 8 natural compounds derived from tocopherol and tocotrienol alpha, beta, gamma, and delta. Alpha-tocopherol is the most abundant form of vitamin E, which exists in cellular membrane and cytoplasmic organelles membrane of human body and acts as fat-soluble antioxidant.<sup>[17]</sup> Vitamin E as a potent and fat-soluble antioxidant, which inhibits production of ROS during fats oxidation and destroys free radicals in biologic membranes,<sup>[18]</sup> is considered a new treatment strategy whose favorable effects on neurodegenerative diseases like Alzheimer's have been proved.<sup>[15,19]</sup>

Regarding the foregoing on the oxidative and apoptotic effects of hypercholesterolemia and also antioxidant and neuroprotective effects of vitamin E,<sup>[20]</sup> this study was conducted to examine the effect of vitamin E on neuronal apoptosis in hippocampal dentate gyrus in rabbits fed with high-cholesterol diets.

## MATERIALS AND METHODS

The studied animals were New Zealand white male rabbits weighing  $2200 \pm 200$  g, which were kept under 12-hour dark/light cycles and unlimited access

to water and food. All the methods used in this study were approved by the Ethics Committee of Isfahan University of Medical Sciences. Before the start of diet, level of serum cholesterol, LDL, and HDL of rabbits was measured. Then, rabbits were randomly divided into 4 groups (each with 6 rabbits;  $n = 6$ ) called control group (fed with ordinary foods), control group (fed with ordinary foods) along with vitamin E (50 mg/kg by Gavage feeding),<sup>[20-23]</sup> high-cholesterol diet group (food containing 2% of cholesterol),<sup>[24]</sup> and high-cholesterol diet group (food containing 2% of cholesterol) along with vitamin E (50 mg/kg by Gavage feeding).

Each group received its own diet for 6 weeks. Groups which were not receiving vitamin E were given saline by Gavage feeding. Then, level of serum cholesterol, LDL, and HDL of rabbits was measured again. Rabbits were anesthetized and after decapitation, their skulls were dissected and the brains were removed and buffered in 10% formalin after fixation, and the tissue passage was molded in paraffin. After molding the tissue sample (hippocampus), coronal cuts of the sample were prepared by cutting through the sample from the beginning to the end into 5 $\mu$  cuts (for hematoxylin and eosin staining) and 3-4 $\mu$  cuts for immunohistochemical staining (TUNEL Test). The slices were placed in an oven for 30-120 min at 56-58 °C in order that the paraffin of the tissue sections can be melted and the tissue well stuck on the slide and not being isolated during staining. Then, diagnostic kits of Roch Co. were used to detect the apoptotic cells based on the instructions of the kits manufacturer. A short time after removal of paraffin, the cuts were treated with proteinase K and incubated for 30 min at 37°C. Then, the cuts were washed with PBS solution (at 25°C) and placed in a solution containing 0.1 mmol of tris-HCl for 30 min at pH of 7 at room temperature, and then, they were washed with PBS solution again. The reaction compound of TUNEL was added to the cuts, and then, they were incubated in a wet chamber for 60 min at 37°C. After that, the cuts were rinsed and diaminobenzidine (DAB) was added to them for 15-30 min at room temperature. Then, they were rinsed with water and stained using hematoxylin. The cuts were cleared in xylol. Then, slides were pasted over the cuts by antillen adhesive and pressed a little to be stuck without air bubbles. Once the slides were cleaned and their adhesive dried, they were prepared to be studied by an optic microscope. Then, images were taken from the samples using a camera attached to the microscope and Motic Image software, and the images were used for cell counting.

Histological results were analyzed using Kruskal-Wallis Test (non-parametric ANOVA) and Dunn's Multiple Comparisons Test for post-test. The results

on the level of serum cholesterol, LDL, and HDL were analyzed using repeated measures ANOVA and Tukey for post-test.

**RESULTS**

Comparison of the results on the level of serum cholesterol, LDL, and HDL among groups showed that the amount of this factor before starting diets was the same in all groups. However, after receiving high-cholesterol diets for 6 weeks, the level of serum cholesterol, LDL, and HDL in the high-cholesterol diet group and high-cholesterol + vitamin E diet group remarkably increased compared to those before starting high-cholesterol diet (intra-group comparison) and those of control groups receiving ordinary diet (inter-group comparison). Furthermore, consuming vitamin E in high-cholesterol + vitamin E diet group insignificantly resulted in a partial reduction of serum levels of above factors [Figures 1-3].

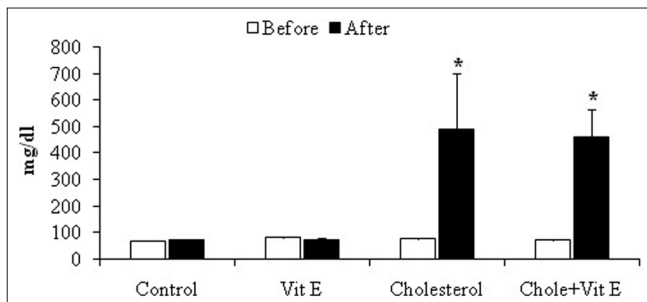
Histological results showed that cholesterol considerably increased neuronal death rate in the granular layer of hippocampal dentate gyrus ( $P < 0.05$ ). However, consumption of vitamin E reduced neuronal death rate in this area of the brain as there was no significant difference between high-cholesterol + vitamin E group

and the control group [Figures 4 and 5].

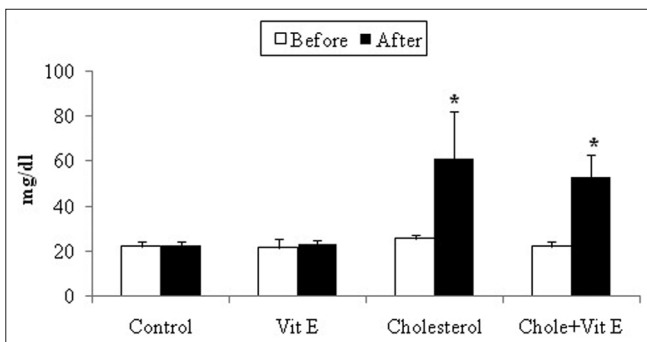
**DISCUSSIONS**

The results of the present study in agreement with previous studies<sup>[13]</sup> showed that consumption of too much cholesterol may lead to increased apoptosis and neuronal death in the granular layer of hippocampal dentate gyrus of rabbits.

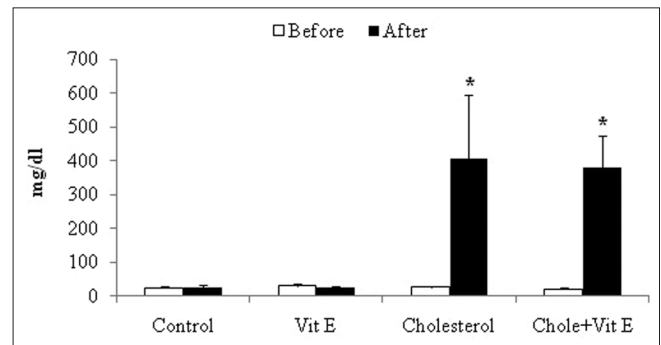
Cholesterol is an important fat having an obvious role in mammalian cell membranes signaling<sup>[16,25]</sup> and neuronal membrane fluidity, although its increase results in the stiffness and in reduction of membrane fluidity. These conditions may damage and destroy cells and remainder of the destroyed cells can act antigens and can trigger inflammatory responses.<sup>[19]</sup> As the blood-brain barrier is highly impermeable to lipoproteins-carrying cholesterol in peripheral blood circulation, cholesterol is produced in brain.<sup>[26]</sup> However, 27-hydroxycholesterol, which is the product of cholesterol oxidation, crosses through the blood-brain barrier<sup>[27]</sup> and with respect to hypercholesterolemia in which the probability of



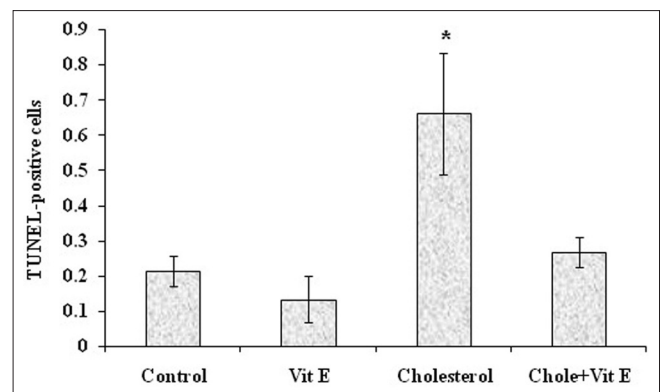
**Figure 1:** Serum cholesterol level in studied groups before and after receiving high-cholesterol diet ( $n = 6$ ,  $*P < 0.05$ , in comparison with the control group after receiving diet, between group comparison)



**Figure 2:** Serum HDL level in studied groups before and after receiving high-cholesterol diet ( $n = 6$ ,  $*P < 0.05$ , in comparison with the control group after receiving diet, between group comparison)



**Figure 3:** Serum LDL level in studied groups before and after receiving high-cholesterol diet ( $n = 6$ ,  $*P < 0.05$ , in comparison with the control group after receiving diet, between group comparison)



**Figure 4:** Rate of neuronal apoptosis in granular layer of hippocampal dentate gyrus of rabbits after receiving high-cholesterol diet ( $n = 6$ ,  $*P < 0.05$ , in comparison with the control group)

influx of 27-hydroxycholesterol increases, the risk for neuronal degeneration increases, too.<sup>[28]</sup> A theory on the course of aging considers it as a result of reactive free radicals. These agents damage different parts of cells such as proteins, fats, carbohydrates, and nucleic acids.<sup>[29]</sup> Among factors, which are highly prone to be induced by RS, are lipid peroxidation and LDL oxidation. Therefore, high plasma level of LDL is a serious risk factor for acceleration of aging.<sup>[30,31]</sup>

It has been found that brain is specifically prone to oxidative injuries due to high use of oxygen<sup>[32]</sup> and the increase in oxygen-free radicals may cause cell destruction and death through oxidation of important parts of cell-like cell membrane and DNA.<sup>[14]</sup> Researchers believe that oxidative stress plays an evident role in formation of protein aggregation in Alzheimer's since the oxidized proteins increase in the brain of patients with Alzheimer's.<sup>[33]</sup>

Hypercholesterolemia is a serious risk factor for induction of oxidative stress in patients with Alzheimer's.<sup>[34]</sup> High consumption of fatty diets causes oxidative stress and inflammation in brain through directly increasing production of oxygen-free radicals, which leads to the increase in production of prostaglandin E<sub>2</sub>, expression of NADPH oxidase subunits, and cyclooxygenases.<sup>[14]</sup> High blood cholesterol increases parameters oxidizing proteins in brain, especially in hippocampus by increasing serum malondialdehyde.<sup>[20]</sup> Moreover, cholesterol increases the production of beta-amyloid peptide, which has an obvious role in pathogenesis of Alzheimer's disease<sup>[35]</sup> and aggregates

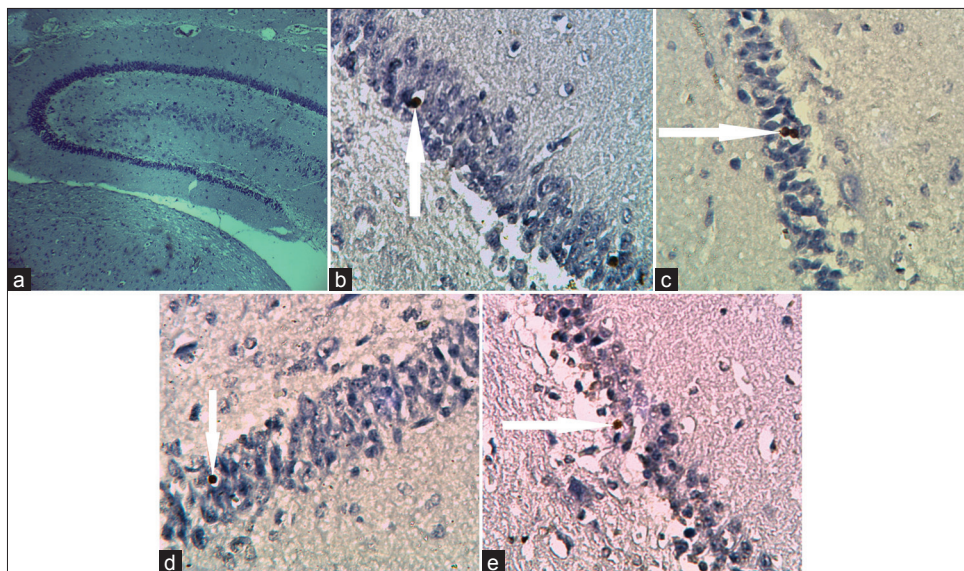
in amyloid plaques along with beta-amyloid fibrils.<sup>[36]</sup>

As a second result, it was shown that although vitamin E did not significantly reduce the level of serum cholesterol, LDL, and HDL in rabbits consuming high-cholesterol, it significantly reduced neuronal apoptosis in these animals.

Based on the above results, disorders caused by hypercholesterolemia are related to oxidative stress.<sup>[10]</sup> Thus, repair of the mechanisms related to oxidative stress equilibrium is effective in prevention and treatment of these disorders. It has been found that vitamin E, which is a fat-soluble antioxidant, inhibits production of ROS during oxidation of fats and destroys free radicals in biologic membranes.<sup>[18]</sup> Alpha-tocopherol is a major antioxidant in lipoproteins and acts as an inhibitor of LDL lipid oxidation.<sup>[37]</sup> This vitamin has been identified to reduce the ability of lipid peroxidation in patients with Alzheimer's.<sup>[38,39]</sup>

Furthermore, tocopherols have strong neuroprotective effects<sup>[37]</sup> and these effects play an important role in prevention of neuronal death not only by elimination of free radicals, but also through non-antioxidant properties like influencing cellular signaling and transcriptional control<sup>[15,40,41]</sup>

Because neuronal death affects aging and is a pathologic ground for many neurodegenerative diseases,<sup>[18,42]</sup> clinical application of vitamin E can be considered as a preventive and treatment strategy in neurodegenerative disorders.



**Figure 5:** Photomicrograph of optical microscope from granular layer of hippocampal dentate gyrus of rabbits (a) (M = x4). Tunel-positive cells in granular layer of the studied groups; control (b), vitamin E (c), cholesterol (d), and cholesterol + vitamin E (e) are visible on the arrow tip (M = x40)

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