Clinical and Experimental Health Sciences

Effect of High Cholesterol Diet and α-Tocopherol Supplementation on Endoplasmic Reticulum Stress and Apoptosis in Hippocampus Tissue

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ABSTRACT

Objective: Neurodegenerative diseases are life-threatening disorders that occur when cells in peripheral nervous system or brain lose function over time and ultimately die. Our study aimed to establish a high cholesterol fed rabbit model to investigate the status of endoplasmic reticulum (ER) stress, proteasome activity and apoptosis as well as the beneficial role of α-tocopherol on hippocampus.

Methods: Hypercholesterolemic rabbit model was established by feeding on 2% cholesterol diet for 8 weeks, and α-tocopherol supplementation was applied with intramuscular injections. Alterations of Grp78, Grp94, Bax, Bcl-2 and BACE1 levels in hippocampus tissue were determined by western blotting, while proteasome activity was measured by fluorometric detection.

Results: Cholesterol containing diet for 8 weeks increased well-known parameters of ER stress and apoptosis, together with no chance in proteasome activity. On the other hand, α-tocopherol supplementation in rabbits fed two percent cholesterol containing diet significantly down-regulated certain ER stress and apoptosis parameters along with an increase in proteasome activity.

Conclusion: Our results underline a novel function of α-tocopherol in reducing cholesterol induced ER stress and apoptosis by increasing proteasome activity in the hippocampus.

Keywords: Hypercholesterolemia, Endoplasmic reticulum stress, Proteasome, Apoptosis, Hippocampus

1. INTRODUCTION

Neurodegenerative diseases are progressive neurologic disorders characterized by neuronal loss and affecting millions of people each year (1). Hypercholesterolemia, a well-defined risk factor for neurodegeneration, is a common feature in general population. (2). Animal studies reported that cholesterol containing diets induce neuronal accumulation of amyloid β (Aβ) and also activates oxidative stress, inflammation and apoptosis signaling (3-5), while clinical studies revealed the positive correlation between plasma cholesterol levels and Alzheimer's disease (AD) (6, 7). Despite all these studies, the underlying mechanisms by which cholesterol causes the development of neurodegenerative diseases remain unclear.

Accumulation of free cholesterol is known to enhance the induction of oxidative and endoplasmic reticulum (ER) stress signalings (8). ER is a crucial organelle in folding of proteins, homeostasis of calcium and signaling mechanisms of cell death. Unfolded protein response (UPR) is a preventive process triggered by ER dysfunction leading to ER stress. UPR induction enhances the ER resident chaperones including glucose-regulated proteins 78 (Grp78) and Grp94 which eliminate misfolded or unfolded proteins (9). Therefore, ER plays a crucial role in neurodegenerative disorders which are defined by misfolded protein accumulation (10). Proteasome has a crucial part in the degradation of intracellular misfolded and damaged proteins. Ability of oxidative stress mediated protein aggregates to inhibit proteasome activity has been identified in many studies and accepted as a primary cause in neurodegenerative disease development (11).

Increased production, oligomerization and aggregation of Aβ plaques formed by the cleavage of amyloid β-precursor protein (APP) by the β-secretase 1 (BACE1) enzyme is one of the major hallmarks of AD (12). Recent studies have shown that ER stress involves in AD development via increasing BACE1 levels (13). However, activation of UPR in the hippocampus suggests the increased presence of Grp78 and Grp94 in AD neurons. Prolonged ER stress developed in neurodegenerative diseases also causes an increase in

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neuronal apoptosis by activation of Bcl-2 family proteins (14). The Bcl-2 protein family consists of proapoptotic (Bax and Bak) and anti-apoptotic (Bcl-2, Bcl-xL and Mcl-1) members. Members of the proapoptotic Bcl-2 family, known as BH3 proteins, activate the intracellular apoptotic pathway in the presence of cellular stress.

Alpha-tocopherol is the most active form of vitamin E. Although the mechanisms of action of α-tocopherol in neurodegenerative diseases are unclear, its critical role in maintaining neurological health has been reported (15). Multiple studies have shown the suppressive role of α-tocopherol in neurodegeneration development via regulating various genes (16-18). For instance, oxidative stress and neuronal cell death in hippocampus tissue were significantly reduced in epileptic rats supplemented with α-tocopherol (19). Another *in vivo* study found that α-tocopherol reduced amyloid oligomers, oxidative stress, and interleukin-1 (IL-1) and IL-6 production (20). α-Tocopherol also acts as a regulator of inflammatory pathways in neuroglia and a cytoprotective factor in hippocampal neurons (21). This literature information support the hypothesis that α-tocopherol might be a critical protective agent in neurodegenerative disorders. However, the effects and mechanisms of α-tocopherol in cholesterol induced brain injury remain poorly understood.

Therefore, the aim of our study is to identify the impact of high cholesterol diet on ER stress, proteasome activity and apoptosis in hippocampus tissue, which are related to pathological hallmarks for neurodegenerative diseases. Furthermore, the influence of a-tocopherol on these signalling was also observed.

2. METHODS

2.1. Rabbits and Treatments

All animal experiments were approved by Marmara University Experimental Animals Research and Implementation Committee (protocol number 49.2015. mar). Starting at 2-3 months old, male albino rabbits were fed with α-tocopherol poor diet or α-tocopherol poor diet containing 2% cholesterol and divided into following groups; i) control, ii) cholesterol, iii) cholesterol + α-tocopherol and iv) α-tocopherol. Intramuscular injections of 50 mg/kg of α-tocopherol (Evigen, Aksu Pharmaceutical) were applied daily to cholesterol+α-tocopherol and α-tocopherol groups. α-Tocopherol concentration used in the study was determined in accordance with our previous studies (22, 23). Rabbits were sacrificed eight weeks after feeding/treatment period, and blood was collected to measure cholesterol and α-tocopherol. Hippocampus was rapidly frozen and stored at – 80°C for immunoblotting.

2.2. Cholesterol and α-Tocopherol Measurement in Serum

Cholesterol levels in serum was identified by using Roche-Hitachi Modular P800 Analyzer. To measure α-tocopherol levels in serum, high-performance liquid chromatography (HPLC) was established following the lipid extraction according to Nierenberg and Nann (24). α-Tocopherol was obtained by C18 column (5µm, 4.6×250mm), while MeOH:dH2O (95:5, v/v) was the mobile phase and a UV detector (Thermo) at 294 nm was used. α-Tocopherol level was calculated as µg/mL following the comparison with the relative peak areas of standard.

2.3. Immunoblot Analysis

Fifteen mg of hippocampus was lysed in RIPA buffer (Cell Signaling) via Ultra Turrax homogenizator in accordance with manufacturer's protocol. After the measurement of total protein amount with Bradford using iMark Microplate Reader (Bio-Rad), twenty µg of protein was loaded onto SDS-PAGE gels, and immobilized on nitrocellulose membrane. After the blocking with 5% skim milk in TBS, membranes were incubated with primary antibodies as follows: Grp94 (Cell Signaling, 2104), Grp78 (Cell Signaling, 3177), Bcl-2 (Cell Signaling, 2870), Bax (Abcam, ab115193), BACE1 (LifeSpan Biosciences Catalog No: LS-A8893-50) and β-actin (Cell Signaling, Catalog No: 4967). Following the HRP-conjugated secondary antibody and chemiluminescence substrate incubations, blots were photographed by ChemiDoc (Bio-Rad) instrument and quantified by Image J.

2.4. Proteasome Activity Measurement by Fluorometry

Proteasome activity measurement was applied according to our previous study (23). Briefly, hippocampus was lysed in 1mM dithiothreitol at 4°C. After the centrifuge at 14000g, supernatants were collected and chymotrypsin-like proteasome activity was determined by using fluorogenic peptide succinyl-LLVY-methyl coumarin as substrate. Following the incubation at 37 °C for 30 min, liberation of methyl coumarin was determined by Enspire 2300 Multilabel Reader (PerkinElmer) at 360 nm excitation and 485 nm emission. Proteasome activity was determined after the comparison with free methyl coumarin as standard. Lactacystin was used in excluding other protease activities.

2.5. Statistical Analysis

Prism 4 (Graph-Pad) software was used in performing statistical analysis. Statistical signifance was estimated using One-Way ANOVA followed Student-Newman-Keuls test for multiple comparisons. P-value less than 0.05 was considered statistically significant.

3. RESULTS

3.1. Cholesterol and α-Tocopherol Levels in Serum

Dietary supplementation of two percent cholesterol in rabbits increased cholesterol levels in serum compared to control (Fig. 1A). Similarly, intramuscular injections of α-tocopherol increased α-tocopherol levels in the serum of cholesterol+α-tocopherol and α-tocopherol rabbits. In accordance with our previous studies (23), α-tocopherol level in serum was

also elevated in cholesterol group, which might be related to increased LDL fraction transporting α-tocopherol in the circulation (Fig. 1B).

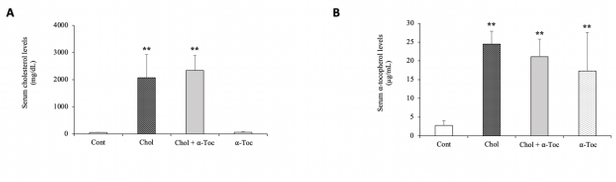

Figure 1. *Serum cholesterol and α-tocopherol levels*
Serum cholesterol (A) and α-tocopherol (B) levels in rabbits receiving eight weeks of 2% cholesterol diet and/or α-tocopherol.
***p < 0.01 vs. control group (n=5).*

3.2. ER Stress and Proteasome Activity in Hippocampus Tissue

After the verification of our *in vivo* model, we next tried to gain insight into the status of ER stress and proteasome. Grp78 and Grp94 are the most well-characterized ER chaperones and well-defined ER stress parameters that participate the correct folding of proteins. In the present study no significant change in Grp94 protein level was observed between groups (Fig. 2A). Despite unchanged Grp94 levels, Grp78 was increased in cholesterol group. Supplementation of α-tocopherol prevented this cholesterol driven Grp78 activation (Fig. 2B). Besides the enhancement of protein folding, activation of protein degradation by proteasome is another process in maintaining protein quality control (25). In this scope, we measured the proteasome activity in rabbit hippocampus tissues by fluorometric detection method. As a potential beneficial effect against high cholesterol induced ER stress, rabbits of cholesterol+α-tocopherol group exhibited significantly increased proteasome activity, while no significant change was determined in α-tocopherol group (Figure 2C).

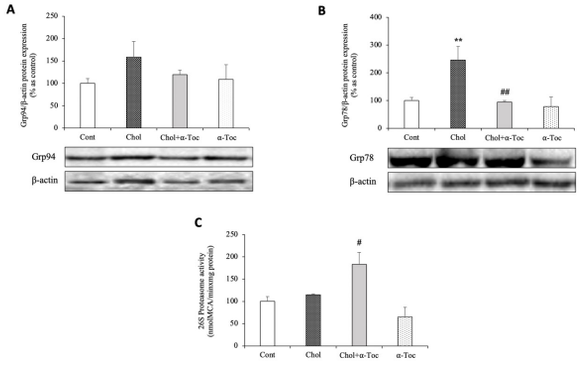

Figure 2. *ER Stress and Proteasome Activity in Hippocampus Tissue*
Hippocampus tissues were homogenized and expressions were measured by western blotting. Protein bands were analyzed and normalized to β-actin against Grp94 (A) and Grp78 (B) antibodies. 26S proteasome activity in hippocampus tissue lysates (C).
Data are expressed as mean ± S.D.
***p < 0.01, vs. control group,*
##p < 0.01, and #p < 0.05 vs. cholesterol group (n=5).

3.3. Bax, Bcl-2 and BACE protein expressions in the hippocampus tissue

As the proteins of Bcl family that induce apoptotic cell death, Bcl-2 and Bax are well identified neuroapoptosis markers (26). Therefore, we determined the levels of Bcl-2 and Bax by immunoblotting. As a parameter of apoptosis induction, high cholesterol diet rabbits elevated Bax and Bcl-2 levels (Figure 3A-B). Alpha-tocopherol supplementation was significantly inhibited the induction of Bax (Figure 3A). We also examined the brain injury in hippocampus tissue by measuring BACE expression. As shown in Fig. 3C, high cholesterol diet induced BACE levels, while α-tocopherol supplementation reversed. We also evaluated the changes in Bax/Bcl-2 ratio and observed a higher Bax/Bcl-2 ratio in cholesterol group compared to control. In agreement with Fig. 3A and 3B, α-tocopherol supplementation reversed this increase (Figure 3D). These results propose the effect of α-tocopherol on high cholesterol diet mediated apoptosis and brain injury in hippocampus tissue.

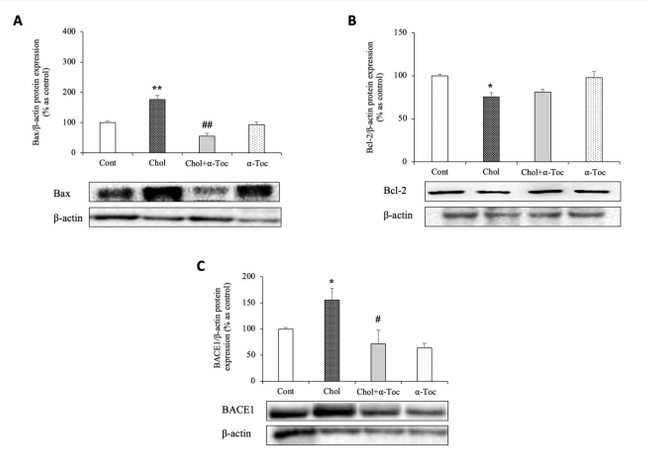

Figure 3. *Bax, Bcl-2 and BACE protein expressions in the hippocampus tissue*
Hippocampus tissues were homogenized and expressions were measured by western blotting. Protein bands were analyzed and normalized to β-actin against Bax (A), Bcl-2 (B) and BACE1 (C) antibodies. Based on the densitometric quantification of the bands for Bcl-2 and Bax, Bax/Bcl-2 ratio was calculated (D).
Data are expressed as mean ± S.D.
***p < 0.01, and *p < 0.05 vs. control group,*
##p < 0.01, and #p < 0.05 vs. cholesterol group (n=5).

4. DISCUSSION

A variety of studies has been reported hypercholesterolemia as a serious risk factor in numerous disorders including neurodegeneration and cardiovascular disease (27). Multiple studies using in-vivo models of AD have found the capacity of high cholesterol diet in increasing Aβ accumulation (28, 29). In this scope, rabbits fed high cholesterol diet exhibited a considerable number of findings of AD similar to those observed in patients with AD such as Aβ accumulation in the hippocampus, apoptosis and microglia activation (30, 31). Therefore, in the present study we established

a hypercholesterolemic rabbit model to determine the protective role of α-tocopherol against ER stress, BACE1 activity and apoptosis in hippocampus. Due to its lipophilic ability, most of the α-tocopherol in serum is bound and carried by circulating LDL and HDL. In our study, elevated α-tocopherol levels determined in serum of cholesterol group are possibly related to its enhanced release from adipose tissue and uptake of LDL fraction (32). However, α-tocopherol in cholesterol group was observed to be reduced by rationing serum α-tocopherol to serum cholesterol, in accordance with our previous studies performed in different rabbit groups (22, 33).

A variety of stress conditions may enhance the misfolded protein accumulation and trigger the ER stress. *In vivo* and clinical findings have determined the interaction between cholesterol and ER stress in the development of metabolic disorders (8). ER contains abundant concentrations of folding sensors and chaperone molecules involved in the quality control process of proteins. Upregulation of Grp78 and Grp94 in brain samples of patients with AD revealed the importance of UPR in disease development (34). Additionally, Lu and colleagues (35) demonstrated the presence of ER stress in the hippocampus of mice fed high cholesterol diet. Similarly, Cai and colleagues (36) determined that rats fed high-fat diet exhibit symptoms of neuronal plasticity together with the induction of hippocampal ER stress. Tunicamycin, an ER stress inducer, is also associated with the induction of Grp78 and Grp94 in cerebral cortex, hippocampus and cerebellum of mice (37). Moreover, Onoda and colleagues (38) revealed that particles derived from air pollution such as carbon black induce misfolded protein accumulation and ER stress in mice brain. In our study, despite the increase of Grp78 in rabbits fed high cholesterol diet, no change was determined in Grp94. This Grp78 increase emphasizes the possible impact of high cholesterol diet in inducing ER stress response and protein damage status. As a response to ER stress, UPR enhances two major degradation systems, proteasome and autophagy, that reduce misfolded/unfolded protein accumulation. Recent studies have determined the reduction of proteasomal activity associated with neurodegenerative diseases that contributes to the failure of misfolded protein degradation and the accumulation of protein aggregates (39). Our data exhibited the increase of proteasome activity in hippocampus tissue when α – tocopherol was supplemented with high cholesterol diet. Thus, α-tocopherol may be effective in the suppression of ER stress through mediating proteasome mediated elimination of damaged proteins.

Uncontrolled and overactivated ER stress in neurodegenerative disorders leads to an increase in neuronal apoptosis, one of the pathological characteristics of neurodegenerative diseases, via activating Bcl-2 family proteins (40, 41). El-Sayyad and colleagues (42) observed an increased neuronal apoptosis in rats fed high cholesterol diet. Increased production and aggregation of Aβ in extracellular area is considered as the major hallmark in AD development. BACE1 is the major enzyme involve in the production of Aβ from amyloid β-precursor protein (APP) which further induces neuronal apoptosis. Related to this, BACE1 protein expression was found to be induced in the majority of AD patients. (43). Zhao and colleagues (14) determined increased protein levels of Bax, Bcl-2 and BACE1 in hippocampus of hyperlipidemic mice. In our study, we observed that the hippocampus of rabbits fed high cholesterol diet exhibited Bax and BACE1 induction. Early studies in animal models were also demonstrated that α-tocopherol is an essential molecule during the development and maintenance of nervous system (44). Accordingly, α-tocopherol has been shown to protect the synaptic plasticity and function by preventing apoptotic cell death in the hippocampus (19). In a study with SH-SY5Y neuroblastoma cells, α-tocopherol was reported to downregulate Bax/Bcl-2 ratio (45). Despite all these studies, the effect of α-tocopherol on cholesterol-mediated apoptosis in brain tissue is unknown. In the present study, Bax and BACE1, two well-studied parameters of apoptosis and AD, were reduced by α-tocopherol supplementation in hippocampus tissue. Our findings suggested that alpha tocopherol may be important molecule in the development of therapeutic strategies against neurodegenerative disorders by targeting apoptotic cell death and BACE1 levels.

5. CONCLUSION

In conclusion, we demonstrate an *in vivo* evidence that the ER stress status and apoptotic activity in hippocampus is likely to be induced in rabbits fed high cholesterol diet. Our results identified the beneficial role of α-tocopherol in cholesterol induced ER stress and apoptosis, possibly via enhancing proteasome mediated degradation of damaged proteins. However, further studies are needed to determine the underlying mechanisms that enhance cholesterol mediated ER stress activation, and the molecules in which α-tocopherol increase proteasome activity in hippocampus.

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Conflict of Interest

The authors declare no conflicts of interests.

REFERENCES

[1] Lee D, Choi YH, Seo J, Kim JK, Lee SB. Discovery of new epigenomics-based biomarkers and the early diagnosis of neurodegenerative diseases. Ageing Res Rev. 2020;61:101069.

[2] Chong AJ, Lim SW, Lee YL, Chio CC, Chang CH, Kuo JR. The Neuroprotective effects of simvastatin on high cholesterol following traumatic brain injury in rats. World Neurosurg. 2019;132:e99-e108.

[3] Sparks DL, Kuo YM, Roher A, Martin T, Lukas RJ. Alterations of Alzheimer's disease in the cholesterol-fed rabbit, including vascular inflammation. Preliminary observations. Ann N Y Acad Sci. 2000;903:335-344.

[4] Refolo LM, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint GS. Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. Neurobiol Dis. 2000;7(4):321-331.

[5] Levin-Allerhand JA, Lominska CE, Smith JD. Increased amyloid – levels in APPSWE transgenic mice treated chronically with a physiological high-fat high-cholesterol diet. J Nutr Health Aging. 2002;6(5):315-319.

[6] Hottman DA, Chernick D, Cheng S, Wang Z, Li L. HDL and cognition in neurodegenerative disorders. Neurobiol Dis. 2014;72 Pt A:22-36.

[7] Popp J, Meichsner S, Kolsch H, Lewczuk P, Maier W, Kornhuber J. Cerebral and extracerebral cholesterol metabolism and CSF markers of Alzheimer's disease. Biochem Pharmacol. 2013;86(1):37-42.

[8] Sozen E, Ozer NK. Impact of high cholesterol and endoplasmic reticulum stress on metabolic diseases: An updated mini-review. Redox Biol. 2017;12:456-461.

[9] Xiang C, Wang Y, Zhang H, Han F. The role of endoplasmic reticulum stress in neurodegenerative disease. Apoptosis. 2017;22(1):1-26.

[10] Lindholm D, Wootz H, Korhonen L. ER stress and neurodegenerative diseases. Cell Death Differ. 2006;13(3):385-392.

[11] Thibaudeau TA, Anderson RT, Smith DM. A common mechanism of proteasome impairment by neurodegenerative disease-associated oligomers. Nat Commun. 2018;9(1):1097.

[12] De Strooper B. Proteases and proteolysis in Alzheimer disease: a multifactorial view on the disease process. Physiol Rev. 2010;90(2):465-494.

[13] Salminen A, Kauppinen A, Suuronen T, Kaarniranta K, Ojala J. ER stress in Alzheimer's disease: a novel neuronal trigger for inflammation and Alzheimer's pathology. J Neuroinflammation. 2009;6:41.

[14] Zhao XS, Wu Q, Peng J, Pan LH, Ren Z, Liu HT. Hyperlipidemia-induced apoptosis of hippocampal neurons in apoE(-/-) mice may be associated with increased PCSK9 expression. Mol Med Rep. 2017;15(2):712-718.

[15] Icer MA, Arslan N, Gezmen-Karadag M. Effects of vitamin E on neurodegenerative diseases: an update. Acta Neurobiol Exp (Wars). 2021;81(1):21-33.

[16] Miyake Y, Tanaka K, Fukushima W, Sasaki S, Kiyohara C, Tsuboi Y. Dietary intake of metals and risk of Parkinson's disease: a case-control study in Japan. J Neurol Sci. 2011;306(1-2):98-102.

[17] Hughes KC, Gao X, Kim IY, Rimm EB, Wang M, Weisskopf MG. Intake of antioxidant vitamins and risk of Parkinson's disease. Mov Disord. 2016;31(12):1909-1914.

[18] Yang F, Wolk A, Hakansson N, Pedersen NL, Wirdefeldt K. Dietary antioxidants and risk of Parkinson's disease in two population-based cohorts. Mov Disord. 2017;32(11):1631-1636.

[19] Ambrogini P, Minelli A, Galati C, Betti M, Lattanzi D, Ciffolilli S. Post-seizure alpha-tocopherol treatment decreases neuroinflammation and neuronal degeneration induced by status epilepticus in rat hippocampus. Mol Neurobiol. 2014;50(1):246-256.

[20] Wang WY, Tan MS, Yu JT, Tan L. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. Ann Transl Med. 2015;3(10):136.

[21] Galli F, Azzi A, Birringer M, Cook-Mills JM, Eggersdorfer M, Frank J. Vitamin E: Emerging aspects and new directions. Free Radic Biol Med. 2017;102:16-36.

[22] Sirikci O, Ozer NK, Azzi A. Dietary cholesterol-induced changes of protein kinase C and the effect of vitamin E in rabbit aortic smooth muscle cells. Atherosclerosis. 1996;126(2):253-263.

[23] Sozen E, Karademir B, Yazgan B, Bozaykut P, Ozer NK. Potential role of proteasome on c-jun related signaling in hypercholesterolemia induced atherosclerosis. Redox Biol. 2014;2:732-738.

[24] Nierenberg DW, Nann SL. A method for determining concentrations of retinol, tocopherol, and five carotenoids in human plasma and tissue samples. Am J Clin Nutr. 1992;56(2):417-426.

[25] Sozen E, Karademir B, Ozer NK. Basic mechanisms in endoplasmic reticulum stress and relation to cardiovascular diseases. Free Radic Biol Med. 2015;78:30-41.

[26] Wang Q, Zhang L, Yuan X, Ou Y, Zhu X, Cheng Z. The Relationship between the Bcl-2/Bax Proteins and the Mitochondria-Mediated Apoptosis Pathway in the Differentiation of Adipose-Derived Stromal Cells into Neurons. PLoS One. 2016;11(10):e0163327.

[27] Brooks SW, Dykes AC, Schreurs BG. A high-cholesterol diet increases 27-hydroxycholesterol and modifies estrogen receptor expression and neurodegeneration in rabbit hippocampus. J Alzheimers Dis. 2017;56(1):185-196.

[28] Ghribi O, Larsen B, Schrag M, Herman MM. High cholesterol content in neurons increases BACE, beta-amyloid, and phosphorylated tau levels in rabbit hippocampus. Exp Neurol. 2006;200(2):460-467.

[29] Jaya Prasanthi RP, Schommer E, Thomasson S, Thompson A, Feist G, Ghribi O. Regulation of beta-amyloid levels in the brain of cholesterol-fed rabbit, a model system for sporadic Alzheimer's disease. Mech Ageing Dev. 2008;129(11):649-655.

[30] Prasanthi JR, Dasari B, Marwarha G, Larson T, Chen X, Geiger JD. Caffeine protects against oxidative stress and Alzheimer's disease-like pathology in rabbit hippocampus induced by cholesterol-enriched diet. Free Radic Biol Med. 2010;49(7):1212-1220.

[31] Deci S, Lemieux SK, Smith-Bell CA, Sparks DL, Schreurs BG. Cholesterol increases ventricular volume in a rabbit model of Alzheimer's disease. J Alzheimers Dis. 2012;29(2):283-292.

[32] Ricciarelli R, Zingg JM, Azzi A. Vitamin E: Protective role of a Janus molecule. FASEB J. 2001;15(13):2314-2325.

[33] Aytan N, Jung T, Tamturk F, Grune T, Kartal-Ozer N. Oxidative stress related changes in the brain of hypercholesterolemic rabbits. Biofactors. 2008;33(3):225-236.

[34] Hoozemans JJ, Veerhuis R, Van Haastert ES, Rozemuller JM, Baas F, Eikelenboom P. The unfolded protein response is activated in Alzheimer's disease. Acta Neuropathol. 2005;110(2):165-172.

[35] Lu J, Wu DM, Hu B, Cheng W, Zheng YL, Zhang ZF. Chronic administration of troxerutin protects mouse brain against D-galactose-induced impairment of cholinergic system. Neurobiol Learn Mem. 2010;93(2):157-164.

[36] Cai M, Wang H, Li JJ, Zhang YL, Xin L, Li F. The signaling mechanisms of hippocampal endoplasmic reticulum stress affecting neuronal plasticity-related protein levels in high fat diet-induced obese rats and the regulation of aerobic exercise. Brain Behav Immun. 2016;57:347-359.

[37] Wang H, Wang X, Ke ZJ, Comer AL, Xu M, Frank JA. Tunicamycin-induced unfolded protein response in the developing mouse brain. Toxicol Appl Pharmacol. 2015;283(3):157-167.

[38] Onoda A, Kawasaki T, Tsukiyama K, Takeda K, Umezawa M. Carbon nanoparticles induce endoplasmic reticulum stress around blood vessels with accumulation of misfolded proteins in the developing brain of offspring. Sci Rep. 2020;10(1):10028.

[39] Ciechanover A, Kwon YT. Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. Exp Mol Med. 2015;47:e147.

[40] Remondelli P, Renna M. The endoplasmic reticulum unfolded protein response in neurodegenerative disorders and its potential therapeutic significance. Front Mol Neurosci. 2017;10:187.

[41] Chi H, Chang HY, Sang TK. Neuronal cell death mechanisms in major neurodegenerative diseases. Int J Mol Sci. 2018;19(10):3082.

[42] El-Sayyad HI, El-Sherbiny MA, Sobh MA, Abou-El-Naga AM, Ibrahim MA, Mousa SA. Protective effects of Morus alba leaves extract on ocular functions of pups from diabetic and hypercholesterolemic mother rats. Int J Biol Sci. 2011;7(6):715-728.

[43] Tan JL, Li QX, Ciccotosto GD, Crouch PJ, Culvenor JG, White AR. Mild oxidative stress induces redistribution of BACE1 in non-apoptotic conditions and promotes the amyloidogenic processing of Alzheimer's disease amyloid precursor protein. PLoS One. 2013;8(4):e61246.

[44] Hill KE, Montine TJ, Motley AK, Li X, May JM, Burk RF. Combined deficiency of vitamins E and C causes paralysis and death in guinea pigs. Am J Clin Nutr. 2003;77(6):1484-1488.

[45] Pahrudin Arrozi A, Shukri SNS, Wan Ngah WZ, Mohd Yusof YA, Ahmad Damanhuri MH, Jaafar F. Comparative effects of alpha – and gamma-tocopherol on mitochondrial functions in Alzheimer's Disease in vitro model. Sci Rep. 2020;10(1):8962.

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