Effect of Atorvastatin and *Lactobacillus acidophilus* on cholesterol metabolism in experimental hypercholesterolemia

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ABSTRACT

Hypercholesterolemia is a very common health problem in the world. In this study, it was aimed to investigate the effects of atorvastatin and *Lactobacillus acidophilus* probiotic on cholesterol metabolism, and formation of neurosterides and myelin. Five groups were formed in the study. Group-1 was fed with standard rat chow as a control group. Group-2 was designated as hypercholosterolemi group and fed with cholesterol added rat chow. Group-3 was fed with cholesterol and atorvastatin. *L.acidophilus* probiotic was given in the last four weeks of the experiment to Group-4. *L.acidophilus* and atorvastatin were given together for the last four weeks to the Group-5. At the end of the trial, some biochemical parameters were determined by autoanalyzer device and ELISA. LDL receptor (LDL-R), HMG-CoA reductase, GAPDH genes were determined by RT-PCR. In the case of adding cholesterol to the diet, total cholesterol in the serum, LDL-cholesterol level increased, HDL-cholesterol level decreased, liver enzyme activity increased, Ox-LDL level increased significantly in the brain, testosterone, progesterone, MBP level, nNOS activity were significantly increased. GAPDH gene gave bands at the same intensities in brain and liver of in all groups. When compared with GAPDH, band intensities of the LDL-R and HMG-CoA reductase genes were decreased. It was determined that the hypocholesteric effect of the combination of statin and probiotic is better and neurosterides have a positive contribution to the level of serotonin hormone. As a result, it was concluded that *L. acidophilus* probiotic supplementation with atorvastatin can be recommended as supportive product in the treatment of hypercholesterolemia.

Introduction

Cholesterol is the precursor of cell membrane, myelin structure and oxysterol, and an important structural element of steroid hormones and bile acids. Brain is the richest organ in terms of cholesterol and contains 20% of the body's total cholesterol. 70-80% of the brain cholesterol was provided in the myelin sheath (12). Cholesterol regulates the tight relationship between neurons and glia. Cholesterol is an essential biomolecule in the formation of synapses and dendrites for normal brain development. Also, it is necessary for axonal development (10). In cholesterol deficiency, degeneration of synaptic and dendrite spine, neurotransmitter errors and decreased synaptic flexibilities could be formed. Defects in cholesterol metabolism cause structural and functional diseases in the central nervous system (6). These metabolic diseases, cholesterol biosynthesis, lipid and lipoprotein transport might be affected by different metabolic pathways such as molecules (12, 24). Hypercholesterolemia is a very common health problem in the world. Statin group drugs that inhibit the HMG-CoA enzyme involved in cholesterol synthesis are widely used in the treatment of hypercholesterolemia. Today, while these drugs are used to effectively reduce the level of

cholesterol in the plasma, there is not enough information about neurosteroids (estrogen, progesterone and testosterone) synthesized from cholesterol in the brain and their effects on myelin formation and cholesterol metabolism. Probiotics such as *Lactobacillus acidophilus* and similar lactic acid bacteriae also have a cholesterol-lowering effect.

The purpose of this study was to compare the effects of atorvastatin and *L. acidophilus* probiotic, which are both commonly used to lower the risk of coronary atherosclerosis following experimental hypercholesterolemia, on cholesterol metabolism and changes in serotonin, neurosteroids, nNOs, oxLDL, and MBP in the brain. The LDL receptor (LDL-R) genes, which are involved in absorption, and the HMG-CoA reductase gene, which is involved in cholesterol metabolism, were also examined in the study.

Materials and Methods

Study population and circumstances: The literature datas were considered as a guide to the selection of the animal species. The rat is the species that is most similar to humans in terms of physiology, anatomy, diet, pathology, and metabolism, according to researches done to explore cholesterol (17). G*Power software (version 3.1.9.7) was used for determining the sample size and analysing power in a study by using using a significance level of $\alpha = 0.05$, 80% power, and an effect size of 0.50. Male Sprague-Dawley breed adult rats (n=50, 10-12 weeks-old, weighing 300-550 g) were obtained from Ondokuz Mayis University, Experimental Animals Application and Research Center. During the study, 22±2 °C room temperature, 60% humidity, 12/12 h light/dark environment was provided. During the investigation, ad libitum feedings were implemented for the experimental animals. The University Experimental Animals Application and Research Center administered regular rat food to produce hypercholesterolemia. A commercial feed mill added 2% cholesterol to the normal feed, mixed it thoroughly, and then re-pelleted it. All groups, excluding the control group, received prepared cholesterol meal as needed during the experiment.

Preparation of probiotic suspensions: For the preparation of *L. acidophilus* suspensions, the lyophilized bacteria were diluted with De Man, Rogosa and Sharpe (MRS) broth. To test the bacteria's viability and purity, they were inoculated onto MRS agar. One milliliter of the culture was added to 500 ml of MRS broth after its viability and purity were confirmed, and it was then incubated at 35°C for 48 h. At the end of the incubation period, the suspension was inoculated to three MRS agar and incubated at 35°C for 48 h. Following incubation, the quantity of bacteria in the primary culture was determined by counting the number of bacterial colonies. Based on the calculations, 1X phosphate buffer solution (PBS) was used to suspend 10^{10} cfu/ml of bacteria, and *L. acidophilus* probiotic suspension was utilized as a treatment (33).

Experimental plan: In the research, 5 groups with 10 animals were formed.

Group 1 (control, C): It was fed with standard pellet rat food for 8 weeks.

Group 2 (hypercholesterolemia group, H): 2% cholesterol added and pelleted standard feed was administrated during 8 weeks for forming hypercholesterolemia (32).

Group 3 (hypercholesterolemia+atorvastatin, HA): 2% cholesterol added and pelleted standard feed was administrated during 8 weeks, and in the last 4 weeks of the trial atorvastatin (Ator, Sanovel) (20 mg/kg/day, dissolved in 0.5 ml drinking water) was administered by oral gavage for treatment purposes (4).

Group 4 (hypercholesterolemia+probiotic, HL): 2% cholesterol added and pelleted standard feed was administrated during 8 weeks, and *L. acidophilus* probiotic ($2x10^8$ cfu/ml) was given by oral gavage for therapy in the last 4 weeks of the trial (31, 33).

Group 5 (hypercholesterolemia+atorvastatin+ probiotic, HAL): 2% cholesterol added and pelleted standard feed was administrated during 8 weeks. For the last four weeks of the trial, the combination of *L. acidophilus* probiotic and atorvastatin was given as described in Group 3 and 4.

At the end of eight weeks, after the rats were weighed, 10% ketasol (0.8-1.3ml/kg) and 2% basilazine (2-5 mg/kg) were administered via intraperitoneally. The animals were decapitated after blood was drawn from their hearts. After coagulation of the blood samples, they were centrifuged at 1550 x g for 10 min and their serums were extracted. The serums were divided into aliquots, and stored at -20 °C until used in the analysis. Following the decapitation, liver and brain tissue samples were taken and the tissues were kept at -80 ºC until analyzes.

Extraction from brain tissue samples for ELISA: Brain tissue samples were extracted for using in ELISA kits in accordance with the instructions provided in the kit. To accomplish this, tissues were rinsed in PBS (0.01 mol/L, pH 7.0–7.2) in ice and dried. Use of a scalpel was used to weigh the tissues and cut them into extremely little bits. In a large glass tube, tissue fragments were placed, and PBS containing a protease inhibitor (5 g/ml aprotinin, 1 mM EDTA) was added to dilute the sample to 10 mg tissue/ml. The ultrasonicator was used to sonicate the regenerated tissues five times for 30 sec each. Following ultrasonication, the suspension was centrifuged at 5000 x g for 5 min to get the supernatant. Nanodrop Spectrophotometer was used to measure the quantity of protein in the supernatants (Thermo, 2000). Before being employed in an ELISA assay, extracted brain tissue supernatants were kept at -80°C.

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Determination of some biochemical parameters in sera: Total cholesterol, HDL, LDL, total protein, albumin, ALT, AST amounts in serum were measured by spectrophotometric method using an autoanalyzer device (BS-120 Vet, Mindray).

Determination of Serotonin, Estradiol, Testosterone, Progesterone, Myelin Basic Protein (MBP), Oxidized Low-Density Lipoprotein (Ox-LDL) and Neuronal Nitric Oxide Synthetase (nNOS) levels in whole brain: Serotonin, estradiol, testosterone, progesterone, MBP, nNOS and Ox-LDL levels in whole brain tissue supernatants were quantified by using the rat specific ELISA kits (cat.no. CSB-E13985r CUSABIO; CSB-E05110r CUSABIO; CSB- E05097r CUSABIO; CSB-E07282r CUSABIO; CSB-E08284r CUSABIO; CSB-E07932r CUSABIO; SEA815Ra Cloud-Clone) according to the manufacturer's instructions. A microplate reader (Infinite F50, TECAN) was used for absorbance measurements. The determined OD_{450} values were calculated with the Magellan Standard Tracker (V7-2) software.

Determination of LDL-R, HMG-CoA Reductase, GAPDH genes by Reverse Transcriptase-PCR (RT-PCR): Brain and liver tissue were used to determine LDL-R, HMG-CoA reductase, and GAPDH genes as described previously (16).

Macherey-Nagel brand NucleoSpin® RNA (740955.50) extraction kit was used for RNA extraction from tissues. The extraction process was carried out according to the method reported by the manufacturer. The concentrations of the extracted RNAs were measured spectrophotometrically with a Nano-Drop spectrophotometer and all RNAs were diluted to an equal concentration of 2 mg/ml. The obtained RNAs were stored at -80°C in order not to deteriorate their structure until use.

The extracted RNAs were translated into cDNA using the cDNA synthesis kit (iScript cDNA synthesis kit, Bio-Rad, 170-8891). This process was carried out according to the method specified by the manufacturer. The obtained cDNAs were stored at -80 ºC to be used in the PCR.

The oligonucleotide sequences and expected band sizes of LDL receptor, HMG-CoA reductase, GAPDH genes were presented in Table 1. PCRs were performed with the methods reported by Park et al (33). For the evaluation of the results, the GAPDH gene was taken as a reference and LDL receptor and HMG-CoA reductase mRNA presences and levels were compared among the groups.

Statistical analyzes: SPSS statistical software for Windows (SPSS-PC, SPSS Inc., Chicago, Illinois, USA) was used for statistical analyzes. The differences and relationships among the groups were examined using Pearson correlation tests, Duncan's multiple range analysis, and one-way analysis of variance (ANOVA).

Results

Serum biochemical parameter levels: The mean and standard deviation of serum total cholesterol, HDL, LDL, total protein, albumin, AST and ALT levels of C, H, HA, HL and HAL groups are presented in the Table 2 and Figure 1 (mean ± SE).

Table 1. Oligonucleotide primer sequences used in RT-PCR (16).

Target gene		Oligonucleotide primer sequences	Expected amplicon size (bp)
LDL	F	ATT TTG GAG GAT GAG AAG CAG	931
	R	CAG GGC GGG GAG GTG TGA GAA	
HMG	F	GCG TGC AAA GAC AAT CCT GGA G	245
	R	GTT AGA CCT TGA GAA CCC AAT G	
GAPDH	F	GCC ATC AAC GAC CCC TTC ATT	702
	R	CGC CTG CTT CAC CAC CTT CTT	

Table 2. The mean ± standard deviation (SE) of total cholesterol, HDL, LDL, total protein, ALT, AST measured in serum (the mean ± standard deviation (SE).

Parameter	Group					
	C	H	HA	HL	HAL	P
TP (g/dl)	6.01±0.11^{ab}	6.4±0.27^{b}	5.84± 0.09^{a}	5.96±0.11^{ab}	6.2±0.22^{ab}	0,095
Alb (g/dl)	3.09±0.04	3.15±0.15	3.18±0.03	3.18±0.03	3.15±0.04	0,001
TC (mg/dl)	51.5± 1.93^{a}	77.66±1.72^{b}	61.77±0.9^{c}	55.2±1.26^{a}	52.88±1.13^{a}	0,071
LDL (mg/dl)	5.41±0.44^{a}	22.85±1.55^{b}	15.41±0.81^{c}	9.22±0.99^{d}	6.64±0.02^{ad}	0,316
HDL (mg/dl)	39.23±2.56^{a}	28.28±1.02^{b}	34.44±1.12^{ab}	36.92±0.8^{a}	33.35±1.45^{ab}	0,007
ALT (IU/l)	54.12±2.94^{ab}	65±4.16^{b}	43.68±3.36^{a}	45.53±4^{a}	41.57±7.22^{a}	0,072
AST (IU/l)	146.45±5.73^{ab}	181.53±11.11^{b}	149.98±12.11^{a}	152.29±7.25^{a}	140.74±10.58^{a}	0,27

$^{a, b, c, d}$: The differences between the groups indicated with different letters on the same line are significant (P<0.05).

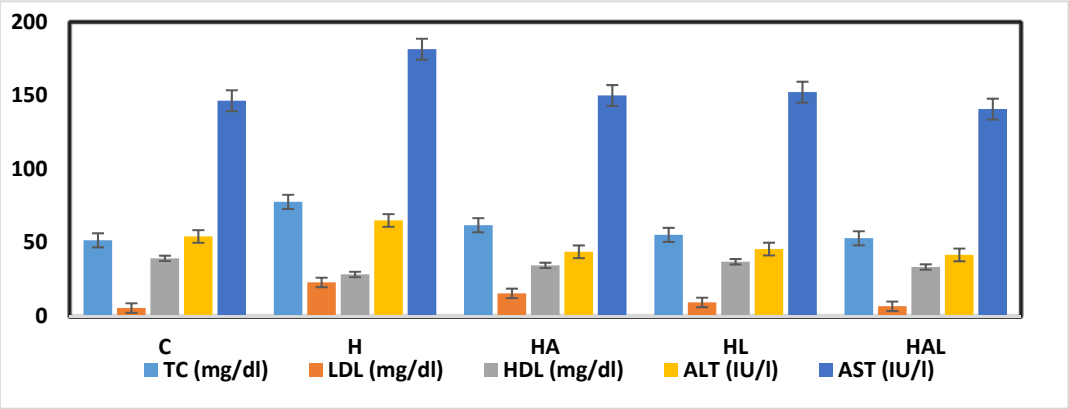

Figure 1. The results of serum biochemistry analyses.
TC: total cholesterol HDL: high density lipoprotein; LDL: low density lipoprotein; ALT: Alanin aminotransferaz; AST: Aspartat aminotransferaz.

Table 3. Correlation of total cholesterol, HDL, LDL, total protein, ALT, AST amounts between groups.

	TP	Alb	HDL	LDL	TC	ALT	AST
TP	1	-0.063	-0.102	0.015	0.177	0.280	0.159
Alb		1	0.003	0.061	0.050	-0.149	0.123
HDL			1	-0.471**	-0.529**	-0.111	-0.213
LDL				1	0.812**	0.376**	0.398**
TC					1	0.356*	0.418**
ALT						1	0.346*
AST							1

*(P<0.05), **(P<0.01).

Serotonin, Estradiol, Testosterone, Progesterone, MBP, Ox-LDL and nNOS levels in whole brain: The amount of serotonin in the H group declined somewhat, began to climb significantly in the HA and HL groups (P>0.05), and increased significantly in the HAL group. The levels of estradiol in the H, HA, HL, and HAL groups were found to be higher than in the C group, and this difference was statistically significant (P<0.05). The testosterone level in the H group was found to be lower than in the C group, and this difference was significant. Progesterone level decreased in the H group compared to the C group, and this decrease was found to be significant (P<0.05). It was determined that the progesterone level increased in HA and HL groups compared to the H group, and this increase was significant (P<0.05). An increase was also found in the HAL group, but it was statistically insignificant (P>0.05). MBP level decreased in H group compared to C group and this decrease was evaluated as significant (P<0.05). A significant difference was determined between the HA and H groups (P>0.05). It was determined that the increase in the HAL group was higher than the HAL group and this was statistically significant (P<0.05). It was determined that the Ox-LDL level increased significantly in the H group compared to the C group, and this increase was significant (P<0.05). Ox-LDL levels were found to be significantly lower in HA, HL and HAL groups compared to C and H groups (P<0.05). The nNOS level was found to be lower in the H group than in the C group, and this decrease was found to be significant (P<0.05). It was determined that the nNOS level decreased significantly in the H and HA groups, and increased significantly in the HL and HAL groups (P<0.05).

Correlation relations: There was a significant positive correlation between serotonin level and estradiol, testosterone, progesterone, MBP and nNOS levels (r=0.779**, r=0.922**, r=0.589**, r=0.618**, r=0.617**, respectively) and Ox-LDL, there was a significant negative correlation (r= -0.506**). A significant positive correlation was found between estradiol level and testosterone, MBP, nNOS (r=0.576**, r=0.629**, r=0.555**, respectively). Significantly positive correlation between testosterone level and progesterone, MBP, nNOS (r=0.684**, r=0.474**, r=0.455**, respectively), and significantly negative correlation with Ox-LDL (r= -0.515*) *) was determined. There was a significant negative correlation (r=-0.465**) between MBP and Ox-LDL. Significantly negative correlation (r=-0.784**) was determined between Ox-LDL and nNOS. The aggregated comparative evaluations of the ELISA results are presented in Tables 3 and 4.

Table 4. Estradiol, testosterone, progesterone, serotonin, nNOS, Ox-LDL levels measured in whole brain tissue extraction (the mean ± standard deviation (SE).

Parameter	Group					
	C	H	HA	HL	HAL	P
Serotonin (ng/mg tissue)	7.5±0.24 [a]	7.34±0.21[a]	15.32±0.69[b]	16.71±0.88[c]	19.43±0.89[d]	0.039
Estradiol (ng/mg tissue)	2.35±0.14[a]	2.63±0.09[b]	2.64±0.11[b]	2.84±0.7[c]	3.01±0.11[d]	0.877
Testosterone (ng/mg tissue)	2.29±0.8[a]	1.34±0.02[b]	4.42±0.12[c]	3.74±0.25[d]	4.62±0.21[e]	0.005
Progesterone (ng/mg tissue)	17.78±0.5[a]	11.64±0.62[b]	23.91±0.51[c]	25.56±0.9[c]	18.14±4.3[a]	0.001
MBP (ng/mg tissue)	2.82±0.24[a]	1.84±0.056[b]	1.63±0.15[b]	3.64±0.32[c]	5.66±0.36[d]	0.028
Ox-LDL (pg/mg tissue)	116.56±1.62[a]	265.09±9.03[b]	197±4.01[c]	106.92±1.27[d]	96.82±0.63[e]	0.001
nNOS (ng/mg tissue)	1.53±0.11[a]	1.34±0.05[b]	1.34±0.03[b]	1.79±0.09[c]	1.88±0.13[d]	0.260

[a, b, c , d, e]: Differences between groups, indicated by different letters on the same line, are significant. (P<0.05).

Table 5. Correlation of estradiol, testosterone, progesterone, serotonin, nNOS, Ox-LDL levels among the groups.

	5-HT	Estradiol	Testosterone	Progesterone	MBP	Ox-LDL	nNOS
5-HT	1	0.779**	0.922**	0.589**	0.618**	-0.506**	0.617**
Estradiol		1	0.576**	0.0229	0.629**	-0.253	0.555**
Testosterone			1	0.684**	0.474**	-0.515**	0.455**
Progesterone				1	0.102	-0.465**	0.277
MBP					1	-0.753**	0.863**
Ox-LDL						1	-0.784**
nNOS							1

**0.001.

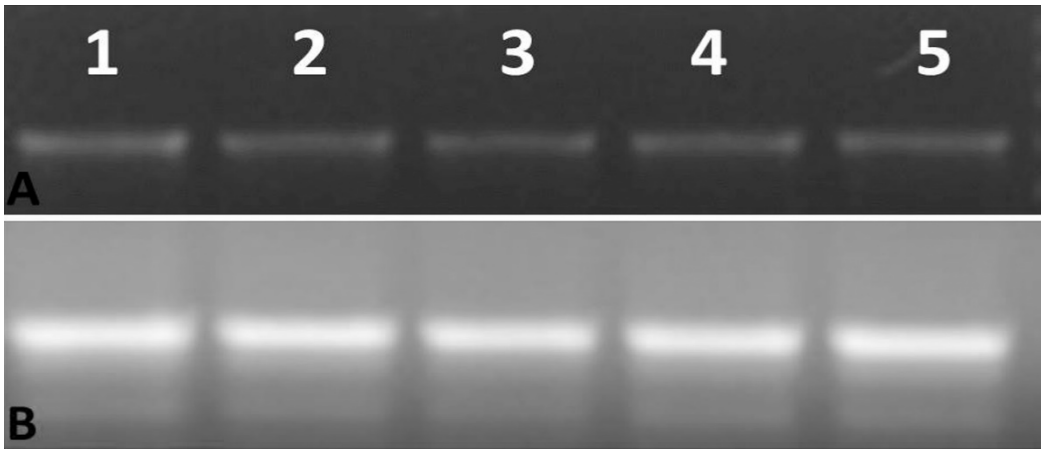

Figure 2. PCR results for the GAPDH gene.
A: brain, B: liver; 1: Group C, 2: Group H, 3: Group HA, 4: Group HL, 5: Group HAL.

Determination of LDL receptor, HMG-CoA reductase, GAPDH by RT-PCR: RT-PCR was performed for determination of LDL receptor, HMG-CoA reductase, GAPDH genes. A 702 bp band was considered positive as a result of PCR performed for the determination of the GAPDH gene. The results obtained with the GAPDH gene were evaluated as the basic profile and the PCR results with other genes were evaluated by considering the intensity of the bands obtained as a result of the PCR for the GAPDH gene. As a result of PCR, it was determined that both brain and liver gave bands at the same intensities in all groups (Figure 2).

A 931 bp band was considered positive for PCR performed to determine the LDL receptor gene. Similar results were obtained as a result of PCR with RNAs extracted from brain and liver. Accordingly, as a result of PCR performed with RNAs from both organ extracts, band intensities were found to be less than that of the GAPDH gene. When the intensities between the groups were compared, the most intense band was determined in the C group. Band intensities were found to decrease as H, HA, HL, and HAL, respectively (Figures 3 and 4).

The presence of a 245 bp band was accepted as positive for the PCR performed to determine the HMG-

CoA reductase gene. Similar results were obtained as a result of PCR with RNAs extracted from brain and liver. Accordingly, as a result of PCR performed with RNAs from both organ extracts, band intensities were found to be less than that of the GAPDH gene. When the intensities between the groups were compared, the most intense band was determined in the HAL group. In the other groups, band intensities were found to decrease as HA, HL, C, and H, respectively (Figures 5 and 6).

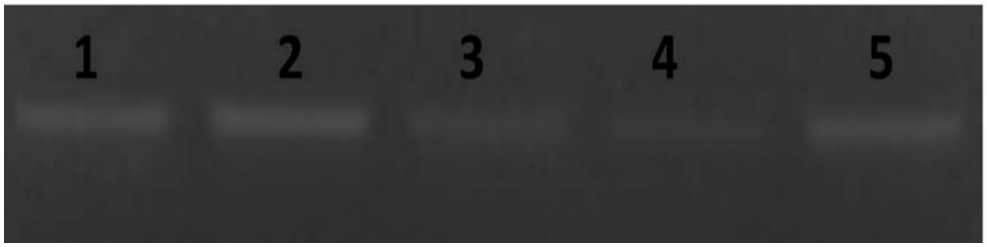

Figure 3. PCR results for LDL receptor gene in brain RNA extracts.
1: Group HA, 2: Group HL, 3: Group C, 4: Group H, 5: Group HAL.

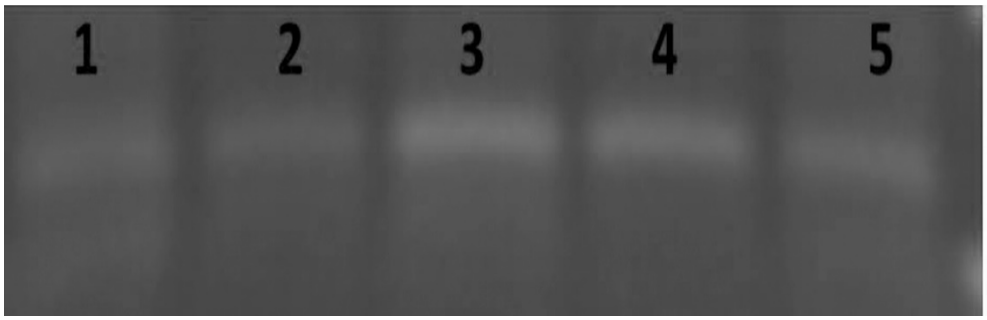

Figure 4. PCR results for LDL receptor gene in liver RNA extracts.
1: Group HA, 2: Group HL, 3: Group C, 4: Group H, 5: Group HAL.

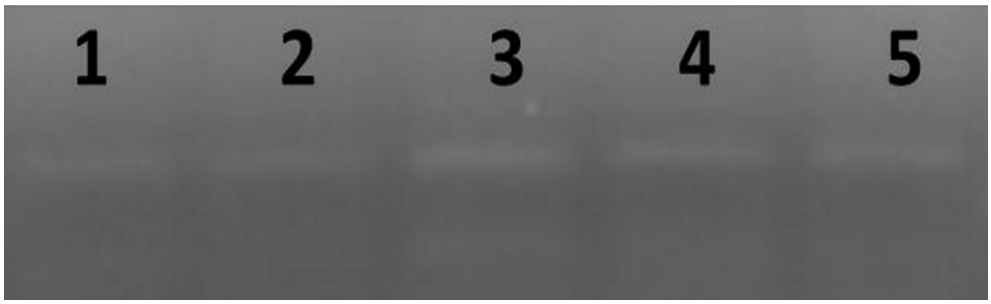

Figure 5. PCR results for HMG-CoA reductase gene in brain RNA extracts.
1: Group H, 2: Group C, 3: Group HL, 4: Group HA, 5: Group HAL.

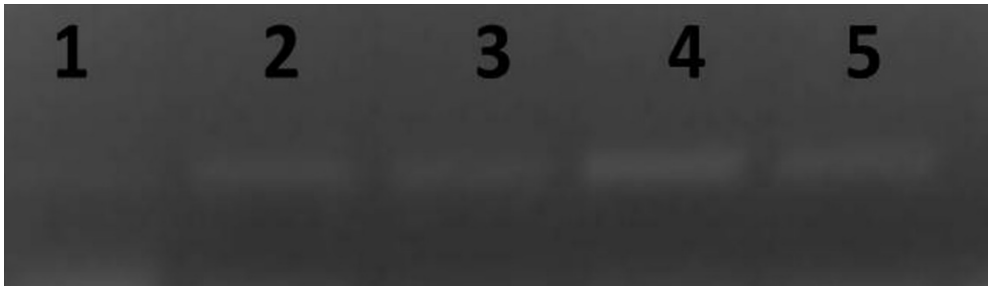

Figure 6. PCR results for HMG-CoA reductase gene in liver RNA extracts.
1: Group H, 2: Group C, 3: Group HL, 4: Group HA, 5: Group HAL.

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Discussion and Conclusion

Cholesterol homeostasis depends on cholesterol biosynthesis, removal of cholesterol from the circulation, dietary cholesterol intake, and excretion of cholesterol through feces and bile. Nutrition plays an important role in the control of cholesterol homeostasis. Cholesterol is essential for the body (an important component of the cell membrane, synthesis of bile acids), absorption of fats and fat-soluble vitamins, precursor in the synthesis of steroid hormones. Eating a high-cholesterol diet is the cause of hypercholesterolemia and cholesterol is also an undesirable culprit, leading to many conditions such as arteriosclerosis and ischemic heart disease, abnormal lipid oxidation or metabolism (32). Cholesterol levels increases in erythrocytes and endothelial cells as well as in serum in hypercholestemia. It has been reported that high cholesterol levels because an increase in oxidized free radical products in these cells (35). Hypercholesterolemia is a common health problem that concerns many people today.

The oxidation of lipids and the increase of reactive oxygen species (ROS), including superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), cause an increase in the amount of Ox-LDL in the cell. Oxidized LDL contributes to endothelial dysfunction by inhibiting nitric oxide (NO) production and also induces proatherogenic genes such as endothelial–leukocyte adhesion molecules and smooth muscle growth factors (37). In our study, total cholesterol and LDL levels in serum and Ox-LDL levels measured in brain tissue extract increased significantly ($P<0.05$), while HDL levels decreased ($P<0.05$) in the hypercholestemic group. It was determined that the hypocholestemic effect was higher in the group given atorvastatin and *L. acidophilus* together with cholesterol feed (HAL) compared to the other groups ($P<0.05$). Anderson et al. (3) found that when *L. acidophilus* contained fermented milk was used for 3 weeks to people with hypercholesteremia, the cholesterol level in the serum decreases by 1% and the risk of coronary heart disease is reduced by 3%. They reported a decrease of 6-10%. Agerbaek et al. (1) detected a significant decrease (3.5%) in serum cholesterol in the consumption of fermented milk containing *Enterococcus faecium* and *Streptococcus thermophilus* probiotics for 3 weeks. Schaafsma (39) reported that when *L. acidophilus* containing yogurt was administreted for 3 weeks, serum cholesterol level decreased by 4.5%. Gilliland and Walker (14) suggested that the bacterial metabolism of *L. acidophilus* contributes to the hypocholestemic effects of cholesterol and bile acids. Harrison and Peat (19) notified the addition of live cultures of *L. acidophilus* to the human intestine may produce a hypocholesterial effect. In the other studies, it was reported that total cholesterol and LDL levels increased significantly, while HDL cholesterol levels decreased in the group that added 1% cholesterol and 0.5% cholic acid to standard rat feed in parallel with our study (2, 18). Administrating the *Cleome arabica* extract (17), lycopene (38), 1% propolis with 1% cholesterol (2), Arginase enzyme (18), *Coptidis rhizome* (38) added groups, it was stated that the cholesterol level decreased and they had a hypocholestemic effect. The activity of the liver enzymes AST and ALT increased in the hypercholestemia group, while liver enzyme activity neared that of the control group in the group administered *L. acidophilus* probiotic and atorvastatin according to our findings (HAL). Hepatic enzymes in hypercholestemic groups were studied concurrently with ours. When lycopene was used, liver enzyme activity neared that of the controland polymethoxylate extract groups (34). In our study, it was indicated that liver enzyme levels were increased in the hypercholestemia group, and AST and ALT enzyme activity was decreased in the group given atorvastatin and *L. acidophilus* together with cholesterol feed for treatment, and these results suggested that it may have a potential protective effect against liver damage.

Cholesterol synthesis inhibitors (CSIs) are known as statins and are used to reduce LDL cholesterol levels (4). Statins, whose main effects are the inhibition of the rate-limiting enzyme HMG-CoA reductase, are also a pleotropic drug with many clinical efficacies (34, 40). Widespread use of statins not only affects CNS cholesterol metabolism, but also has important effects on CNS morphology and neuron functions (40). While *in vitro* studies conducted to better understand the effects of statin use on brain cholesterol metabolism and Alzheimer's disease have found positive associations, there are still contradictions in *in vivo* studies. Statins have positive effects on NO synthesis and endothelial function as well as lowering the lipid level in serum. This effect is caused by the phosphorylation of NO products and endothelial nitric oxide (eNO), resulting in an anti-angiogenic effect. Although the benefits of statins which are cholesterol-lowering drugs, are important on the heart, it has been reported that the progressive effect of the use of statins or the low or decreased cholesterol level may be associated with disorders in cerebral serotonin metabolism, and the drug should be discontinued in psychiatric diseases when there is a change in consciousness, emotion and behavior (aggression and violence).In a study, it was notified that the antidepressant property of atorvastatin is associated with the serotonergic system, and it can be used in alternative treatment by reducing the dose of antidepressant medication (27). In our study, the level of serotonin hormone fell slightly ($P>0.05$) in the hypercholestemic diet (H) group compared to the control group, but increased in the groups given solely

atorvastatin (HA) and only *L. acidophilus* (HL). This rise was shown to be statistically more significant in the group given the *L. acidophilus* probiotic in combination (HAL) ($P<0.05$). Depression and mental illnesses can be caused by low serotonin hormone levels. In our study, it was concluded that co-administration of atorvastatin (HA) and *L. acidophilus* probiotic together with the aim of treating hypercholesemia had a positive effect as an antidepressant by increasing the serotonin hormone level better than giving it separately.

Nitric oxide is a vasodilator produced from L-arginine and plays an important role in regulating the functions of the cardiovascular, nervous and immune systems. There are three isoforms of NO. These are neuronal nitric oxide synthetase (nNOS), endothelial nitric oxide synthetase (eNOS), and inducible nitric oxide synthetase (iNOS). nNOS is intensely synthesized in the cerebellum, and it has been reported that nitric oxide produced plays a neurotransmitter role and causes neurotoxicity due to excessive stimulation of neurons by glutamate (9). It has been reported that nitric oxide products are decreased in response to oxidative stress, one of these complications associated with dyslipidemia (20). Hypercholestemia was increased the NO degradation by causing an increase in oxygen-derived free radicals in the endothelium (29). nNOS is specific for central and peripheral nervous system cells. It was informed that nNOS activity in the brains of patients with schizophrenia and depression was significantly lower than in the control group (5). In our study, nNOS levels decreased in the hypercholestemic diet (H) group compared to the control group, but increased in the hypercholestemic diet with *L. acidophilus* (HL) group, and this increase was statistically more significant in the group given *L. acidophilus* probiotic along with atorvastatin (HAL). It was concluded that taking atorvastatin and a probiotic like *L. acidophilus* at the same time can help prevent oxidative damage in hypercholesterolemia and neurological diseases by increasing vascular blood flow in the central nervous system and removing unwanted radicals and amyloid deposits from the environment.

Cholesterol is an integral part of myelin. The myelin layer consists oligodendrocytes and schwan cells. Oligodendrocytes surround neurons in the peripheral and central nervous system. Myelin layers are compact monolayers of Schwann cells and oligodendrocytes that form around the axons of neurons in the peripheral and central nervous system (CNS), respectively. It has been stated that demyelination and consequent deterioration of signal transmission can lead to severe neurological disorders and multiple sclerosis (MS) by causing focal deposition of cholesteryl esters in the brain and disruption of myelin structure. Some steroids are synthesized in the nervous system independently of peripheral endocrine glands and are named as 'neurosteroids'. The synthesis of neurosteroids in the nervous system starts from cholesterol. Experimental animal studies show that steroid synthesis in the CNS affects many functions of the brain and regulates protein synthesis through direct transcriptional changes via intracellular receptors (5). Neurosteroids were identified for the first time in male rat brain and reported as DHEA, pregnenolone and their sulfate esters (6). Other steroid hormones (estradiol and testosterone) were also detected in the brain of adult mammals (7). When neurosteroids are given to animals, they mostly show anticonvulsant, myorelaxant, anesthetic and anxiolytic effects (12). Estrogen, progestin, androgens, progesterone and dehydroepiandrosterone affect neuron function and play an important role in the nervous system, especially in aging (24). The levels of testosterone and progesterone hormones in brain tissue were found to be substantially lower ($P<0.05$) in the hypercholestemic diet group (H) compared to the control group in our study. The group given only atorvastatin (HA) and alone *L. acidophilus* (HL) with a hypercholestemic diet had a rise in testosterone levels, and this increase was statistically more significant in the group given atorvastatin and *L. acidophilus* probiotic combination ($P<0.05$). It was determined that the level of progesterone hormone in the brain decreased in the H group compared to the control group, and increased in the group treated with Atorvastatin (HA) and *L. acidophilus* (HL), this increase was greater than when given together (HAL).

Estradiol is produced by crossing the blood-brain barrier and endogenously from cholesterol in the brain. In addition to have an important role in the physiological and reproductive system, estradiol has been shown to be an important signaling molecule in the brain in recent studies. Estrogen has been shown to have beneficial effects on the entire bioenergetic system of the brain for the transport of glucose into the cell, glycolysis, tricarboxylic citric acid cycle, oxidative phosphorylation and ATP production (7). *In vitro* studies have shown that estrogen protects against DNA damage induced by hydrogen peroxide and arachidonic acid (28). In our study, estradiol level increased slightly in rats fed a hypercholestemic diet compared to the control group, and there was a significant increase in the group given only atorvastatin (HA) and only *L. acidophilus* (HL), and this increase was statistically significant in the group given atorvastatin and *L. acidophilus* probiotic together (HAL) was determined to be more important ($P<0.05$). It has been reported that estrogen in the central nervous system protects against the accumulation of beta amyloid proteins in neurons and increases glucose utilization by increasing cerebral blood

flow (36). These results suggested that the combined use of atorvastatin and *L. acidophilus* in hypercholestemic patients may be effective in reducing neurological diseases such as Alzheimer's diseases. It has been notified that when the total cholesterol and LDL cholesterol increase in serum, LDL-R protein expression decreases when fed with a high-cholesterol diet (26). It has also been informed that a high-fat and cholesterol diet reduces LDL-R mRNA in the liver. It has been stated that there is a relationship between LDL receptor activity and the amount of LDL receptor protein, and that lack of LDL-R in rats may contribute to the increase in serum total cholesterol and LDL cholesterol (21). In most of the studies in the literature, it was reported that the use of lipodemic drugs affected LDLr expression (13). In another study with a hypercholestemic rat model, LDL-receptor was examined and liver LDL-R protein expression was found to be significantly decreased in the high cholesterol group compared to the other groups (22). Lactic acid bacteria are known to lower the cholesterol by increasing the excretion of bile acids. It has been reported that the reason why some *Lactobacillus* species lower cholesterol may be due to their collapse by not being able to bind with bile salts (25). Bile salt hydrolase is the enzyme that converts free bile acids into deconjugated form. It has been reported that the loss of bile salts with feces can reduce its level by using cholesterol for the formation of new bile salts (41). In a study, it was reported that after adding *Lactobacillus plantarum* to the control group, liver LDL receptor expression was affected and increased compared to the control group (23). In our study, the most intense band was determined in the HL group as a result of RT-PCR performed to determine the presence and level of LDLr mRNA. In the other groups, band intensities were found to be decreased as HAL, HA, C and H, respectively. The result obtained is in parallel with the studies in the literature, and it was determined that LDLr mRNA was at a higher level in rats fed with drugs and probiotic supplements used to reduce cholesterol levels. Goldstein and Brown (15) stated in their study using the northern blot method that when cholesterol is high, the amount of LDLr mRNA decreases and LDLr expression is regulated by negative recycling of cholesterol. As a result of the increase in cholesterol concentration in the cell, LDL cholesterol is released into the plasma, and as a result, it has been reported that LDLr and mRNA activity decreased in animals fed a high cholesterol diet (11). However; studies with different results are also available in the literature. In a study, it was notified that administration of Polysaccharide from fuzi for treatment in hypercholestemic rats had a reducing effect on LDL-receptor expression in the liver (22). HMG-CoA reductase is a restriction of cholesterol biosynthesis and is a peroxisomal enzyme bound to the endoplasmic reticulum. While this enzyme is expressed in all tissues, its expression level is higher in the liver. It plays a central role in cholesterol synthesis and regulation of cholesterol level in plasma. Ness et al. (30) reported that HMG-CoA reductase was affected very little as a result of feeding with a cholesterol diet, and the addition of cholesterol-lowering substance to the diet increased 15-20 times in both HMG-CoA reductase mRNA and activities. HMG-CoA reductase also has a limiting effect on the mevalonate pathway, which is an important pathway of cholesterol synthesis. In the literature, it has been reported that the inhibition of this enzyme in the liver with statins causes a decrease in cholesterol synthesis, an increase in LDL-R synthesis, and therefore a decrease in LDL and cholesterol levels in the circulation (8). The finding in rats fed a high-cholesterol diet differed from a previous report suggesting that increased cholesterol absorption associated with a high-fat diet resulted in increased free cholesterol levels in hepatocytes and consequent inhibition of HMG-CoA reductase expression. In a study investigating HMG-CoA reductase in a hypercholestemic rat model, HMG-CoA reductase mRNA expression was found to be significantly higher in the high-cholesterol group than in the control group and those treated with FPS (22). Lactic acid bacteria are known to lower cholesterol by increasing the excretion of bile acids. It has been reported that the loss of bile salts with feces can reduce its level by using cholesterol for the formation of new bile salts (41). In a study, it was notified that after the addition of *L. plantarum* to the control group, liver HMG-CoA reductase expression was affected and increased compared to the control group (23). In our study, the most intense band was determined in the HAL group as a result of RT-PCR performed to determine the presence and level of HMG-CoA reductase mRNA. In the other groups, band intensities were found to be decreased as HA, HL, C and H, respectively. As a result, it was determined that HMG-CoA reductase mRNA was higher in rats fed with drugs and probiotic supplements used to reduce cholesterol levels.

In conclusion, in the case of adding 2% cholesterol to the diet, total cholesterol in the serum, LDL-cholesterol level increases, HLD-cholesterol level decreases, liver enzyme activity increases, Ox-LDL level increases significantly in the brain, testosterone, progesterone, proteins in the myelin sheath. The important thing is that the level of MBP, which is expressed as the manager of myelin proteins, and nNOS activity decrease significantly, which may lead to the formation of neurological diseases in the brain. By lowering cholesterol levels better (hypocholestemic) than those in the given group, neurosteroid levels, which have a protective role in the brain, and by increasing the level of the hormone

Serotonin, which has antidepressant properties by increasing its level, neurological diseases shaped by high cholesterol levels in the brain (for example, Alzheimer's) and learning and memory disorders shaped by myelin regulation. It is thought that it can be used in preventive treatment in cases and will shed light on further studies to be done.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

GÇ and AÇ conceived and planned the experiments. AÇ and TG contributed to bacterial sample preparation. GÇ, MÇ, BO, and SÇ carried out the experiments. GÇ and AÇ contributed to the interpretation of the results, and took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical statement

The study was approved by the Animal Experiments Local Ethics Committee of Ondokuz Mayis University (Approval no: 2016/08).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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