The effect of an induced hypercholesteremia on the regulation of the ARC gene expression, Brain Derived Neurotropic Factor (BDNF), Synapsing-1 and neurotransmitter –glutamate in male rats

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Abstract

An abnormality of lipid metabolism known as hypercholesteremia, which causes blood cholesterol levels to be abnormally high, is a significant health concern. This research, in light of its association with neurodegenerative disease, is of paramount importance as it aims to elucidate how elevated cholesterol levels impact glutamate levels, BDNF, Synapsin K, and ARC gene expression. Ten male rats were meticulously assigned to a control group and another group that was given a 1% cholesterol supplement for 28 days. Each group received a random assignment of rats to ensure the validity of the results. Samples of homogenized brain tissue and serum blood were obtained after the experiment's completion, following a meticulous experimental design. Triacylglycerol (TAG), low-density lipoprotein (LDL), and total cholesterol (TC) values rose significantly, whereas HDL and brain-derived neurotrophic factor (BDNF) fell sharply, according to the study's findings. The results indicated that hypercholesteremia had a negative impact on glutamate levels, BDNF, and synapses due to oxidative stress, while synapsin-1 levels in brain tissue remained unchanged. There were also notable decreases in serum and homogenized brain tissue concentrations of NO and GSH, along with increases in MDA. Additionally, there was a noticeable decrease in glutamate levels in brain tissue and down-regulation of the Activity Cytoskeleton Association protein gene (ARC gene) expression.

Keywords: hypercholesteremic diet, glutamate, BDNF, oxidative stress.

Introduction

There are two reservoirs of cholesterol in the brain, one in the myelin membrane and the other in the glial and neuronal plasma membranes. The overall amount of cholesterol in the body is largely comprised of this organ. It is already known that abnormalities in cholesterol metabolism are associated with neurodegenerative diseases [1], and new evidence suggests that cholesterol plays a critical role in synaptic transmission. A key risk factor for neurodegenerative disease development is hypercholesterolemia, which is defined by abnormally high lipid levels or raised plasma cholesterol. For example, conditions like Alzheimer's and cerebrovascular damage [2].Cholesterol levels are highest in the brain, which accounts for over 20% of total body cholesterol [3]. Astrocytes, microglia, and oligodendrocytes are the main cells in the brain that regulate cholesterol. Normal brain function depends on this molecule.The development of synapses and dendrites is dependent on cholesterol [4,5]. Cholesterol metabolism is handled independently by the peripheral nervous system (PNS) and the central nervous system . Cholesterol needs in the central nervous system are satisfied by cholesterol produced locally since plasma lipoproteins cannot cross the blood brain barrier (BBB).Astrocytes secrete specific lipoproteins such Apolipoprotein-E (Apo-E) that carry cholesterol throughout the central nervous system [6,7].The application of the cholesterol-Apo-E complex to the distal end of neurons speeds up axonal extension, but this effect is not observed when applied to the cell body.Events associated with hypercholesterolemia cause harm to the central nervous system [7, 8].There are Numerous signalling events can be triggered by elevated brain cholesterol levels through cholesterol metabolites, pro-inflammatory mediators, and antioxidant mechanisms [9,10,11,12].

A member of the neurotrophin family, Brain-derived neurotrophic factor (BDNF) is highly expressed in the central nervous system.. All of the following neuron growth, regeneration, survival, and maintenance are under its purview [13]. The original function brain-derived neurotrophic factor (BDNF) is to aid in neuronal proliferation, neurogenesis, differentiation, and degeneration, similar to other neurotrophins [14]. Evidence also points to its role in controlling activity-dependent neuronal change [15]. On the inside of synaptic vesicles (SVs) lies a phosphoprotein known as synaptin VII that is unique to neurons. The principal role it plays in presynaptic terminals is to regulate SV dynamics. Synapsin I forms clusters inside the reserve pool when SVs are at rest by interacting with the F-actin cytoskeleton and phospholipids. [16].

Neuronal activity induces the expression of several early genes, including the Arc (activity-regulated cytoskeleton-associated protein) gene. We don't know how the Arc gene expression is regulated.brain-enriched immediate early gene that affects critical pathways implicated in memory and learning is activity-regulated cytoskeleton-associated protein (Arc) [17].

Formation of Memories, storage, and recall all include an elevated arc, which also plays a role in mood regulation and reward-reinforcement [18,19,20]. Aβ oligomers are

discovered to dysregulate Arc; It is connected to a number of neurological diseases, such as Alzheimer's disease [21].

This study aims to determine the effect of hypercholesteremia in male rats given 1% w/w cholesterol every day in their diet for four weeks on the central nervous system and the associations between ARC gene expression, BDNF, synapsin-1, glutamate-1, and other chemical parameters.

Material and Methods

Ethical approval

This study, under the reference number UOK.VET.PH.2022.046 was carried out in the anatomical facility of Kerbela University/College of Veterinary Medicine in Iraq.

Methodology for an experiment

In this study, twenty male rats of white colour, ranging in age from 10 to 14 weeks and weighing 180g±60g, were sourced from the University of Kerbala in Iraq's College of Pharmacy. clean, specialized plastic cages were used to house the animals. The air and surroundings are just right for them. Both the light cycle and the relative humidity were set to 12 hours and 55%, respectively. They were kept for two weeks to give them time to acclimatize to the standard testing environment. The temperature was steady at 22–25 degrees Celsius. The animal was fed pellets of freshly prepared feed while a ventilation vacuum was used to continuously alter the air in the room and a room thermostat.

Experimental Design

Two groups of ten male rats each group were randomly assigned the following treatments. The rats in the first group were fed a standard oral diet for four weeks, while the rats in the second group were given cholesterol 1% (w/w) [18].

Blood collection

Once the animals were sedated and given a chloroform inhalation, sterile medical syringes containing 5 ml were inserted into the heart. The samples were then centrifuged for five minutes at 4000 revolutions per minute in speed in a dedicated gel tube. During the experiments, the serum was chilled to -30 ° C and placed in Eppendorf tubes after separation.

Collecting the brain tissue

Squishers were used to achieve uniform tissue distribution, weighted crushed brain samples for four minutes and used (200 litres) of perchloric acid (0.1 N). After the homogenates were centrifuged cold for 30 minutes, the liquid at the top was gently poured off in a 40C incubator. For analysis, the supernatants should now be refrigerated at -80°C or collected right away. Obtain 500 millilitres of glacial acetic acid that has been dehydrated, 25 millilitres of acetic anhydride, and approximately 8.5 millilitres of perchloric

acid. during the entire day. Anhydrous glacial acetic acid (1000 cc) is added to complete the mixture.

Analysing nitric oxide (NO) concentrations

No metabolite concentration in the supernatant was measured using the Griess reaction as the basis for a colourimetric technique **[22]**, which allowed for an estimation of the amount of NO generation. The results were represented as nanomoles per milligramme of protein and were determined utilizing sodium nitrite in distilled water to create a calibration curve.

Levels of glutathione assessed

The concentration of glutathione in both serum and homogenised brain tissue was measured spectrophotometrically using Ellman's reagent [23]. The GSH concentrations were determined by first precipitating all of the proteins in the tissue homogenate using a precipitating solution. The clear supernatants were collected for analysis after centrifugation for 15 minutes at 4 °C at 11,000 x g.

Monitoring malondialdehyde concentrations

We used homogenised brain tissue and serum of malondialdehyde as indicators of brain tissue and plasma lipid peroxidation, using the procedure from [24].

Determination of tissue Glutamate of brain study to measure MDA levels

Following the scientist's protocol, the amino acids were isolated [25,26]. After weighing, the sample was moved to a 25 ml volumetric vial along with 3 grammes of hydrochloric acid (6M) and left to sit at 150 m for three hours. Following the sample's drying using the rotary evaporator, 5 ml of sodium citrate solution 2.2 pH was introduced. After that, the mixture was transferred to the injection device and filtered via a 0.45um plastic filter.

Analyzing Blood Lipid Parameters

Their results for TC, TAG, LDL, and HDL serum have used the technique in the review [21,22,28] to measure the level.

Gene expression analysis.

This technique employed the comparative Ct method (ΔΔCt) with normalisation to the control group's levels in the presence of transcript levels to those of GAPDH mRNA. This was accomplished in accordance with the suggestion made by [29].

Statistical analysis

$P \leq 0.05$ was selected as the threshold of significance when using the t-test and correlation with the statistics program Graph Pad Prism 8.0. The data points displayed as mean +/- SD.

Results and Discussion

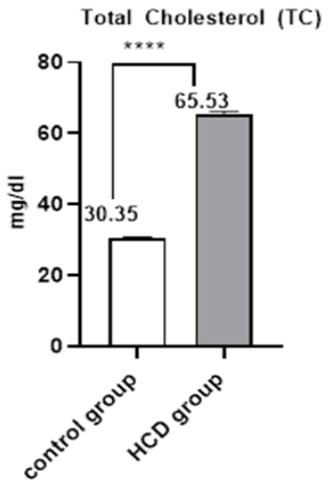
The current study shows a significant ($p \leq 0.0001$) increase in serum TC, TAG and LDL (65.53±0.25, 87.02±0.59, 19.17±0.11) in the hypercholesteremic diet group in contrast to the control groups (30.35 ± 0.16, 39.93±0.18, 11.168±0.21) Figure (1), the HDL level significantly decreasing (24.08±1.27) in the hypercholesteremic diet group contrast with the control groups (37.24±0.24) Figure (1).

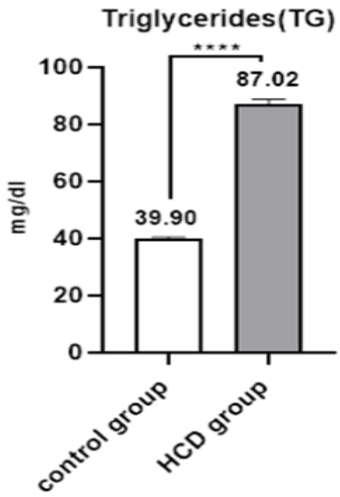
The BDNF level shows a significant ($p \leq 0.0001$) decrease(9.17± 0.28) in the hypercholesteremic diet group in contrast to the group under control (19.42±1.48) Figure **(2)**. respectively, while Figure (3) showed no significant differences in synapsin 1 level (0.41±0.03) in the hypercholesteremic diet group in contrast to the group under control (0.38±0.01).

The gene expression ARC gene shows a significant down-regulation ($p < 0.05$) of the group treated with 1% cholesterol (0.6943 ± 0.06) compared with the control group (1.028 ± 0.114) in Figure (4,5).

The results of the current investigation indicate a significant ($p \leq 0.0001$) decrease in nitric oxide (NO) and GSH (2.78±0.26, 22.07±0.31) with a significant increase in the MDA concentration (7.91±0.17) in the hypercholesteremic diet group in contrast to the group under control (5.86±0.17, 52.36±0.48, 5.84±0.19) as shown in Figures (6 and 7).

Figure (8) demonstrated a significant increase in the serum MDA (0.344 ± 0.008) of the hypercholesteremic diet group as contrast with the control groups (0.142 ± 0.003) and a significant decrease in the GSH concentration (2.90±0.14)in the hypercholesteremic diet group as contrast with the control groups (4.02 ± 0.02).while the brain glutamate show a significant (($p \leq 0.0001$) decrease in the hypercholesteremic diet group (228.07±1.14) in contrast to the group under control (14.3±0.44) in Figure (9).





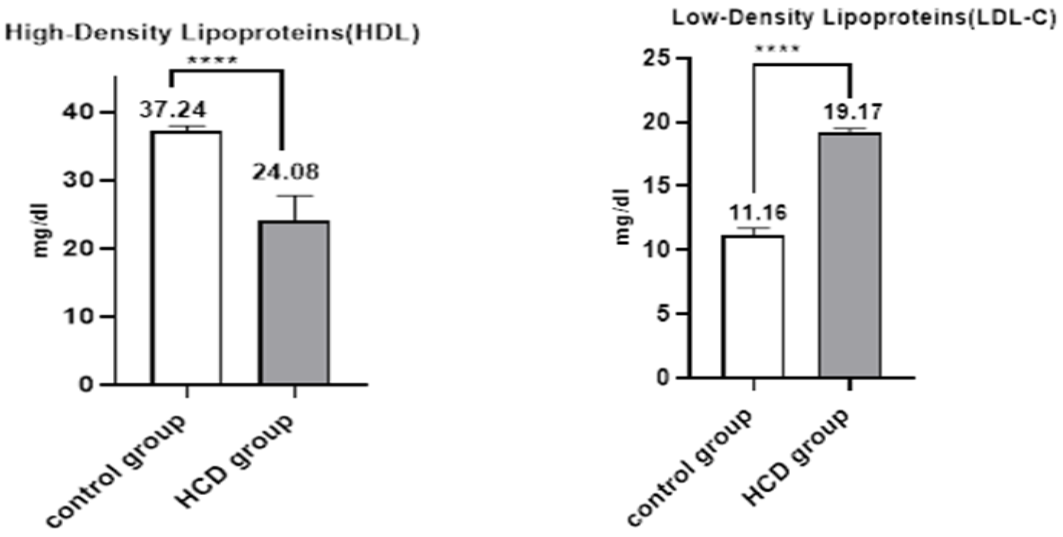


Figure (1): Effects of 1% hypercholestermic diet for 4 weeks on the lipid profile (TC, TAG, LDL-C, and HDL-C) of the serum concentration in the male rats.

Cholesterol is a cellular component that is crucial for cellular homeostasis and transmembrane communication (the movement of information across cell membranes) [25].There are two different types of cholesterol located in the brain. White matter's myelin membranes comprise 70% of the brain's total metabolically stable cholesterol, while Within the grey matter, the plasma and subcellular membranes of neurons and glial cells contain 30% [27].the current study found that adding 1% cholesterol to the diet daily for four weeks improved TC, TAG, and LDL while reducing HDL in group of the cholesterol compared to group of the control [30, 31,32, 33, 34,35].

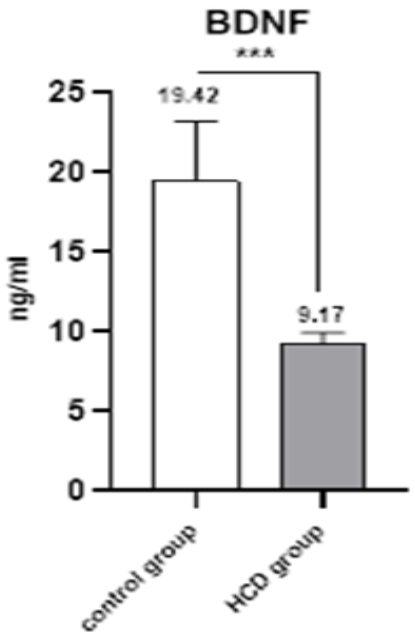


Figure (2): Effects of 1% hypercholesteremic diet for 4 weeks on the serum BDNF concentration in the male rats.

Cholesterol and its oxidised metabolites, called oxysterols, are unable to cross the BBB or blood-brain barrier, but oxysterols can enter the brain and play a vital role in brain regulation cholesterol metabolism [36].

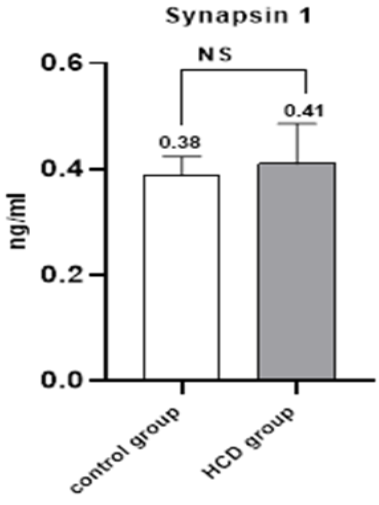


Figure (3): Effect of 1% hypercholesteremic diet for 4 weeks on the serum synapsin 1 concentration in the male rats.

Dysfunctional synapses cause cholesterol to leak out of brain cells, which in turn inhibits long-term potentiation (LTP) [37]. The creation of several cerebrosterols that control neuronal metabolism, as well as synaptic activity, which involves the organisation of proteins in the synaptic bouton, both require cholesterol [37, 38, 30]. An age-related decline in neuronal cholesterol levels may play a significant role in synapse loss [39] and, ultimately, neuronal death [40]. The high cholesterol level in other brain regions, such the myelin sheath, makes it difficult to examine brain cholesterol fluctuations. However, there are significant implications for neuro-degeneration that might be explored by analysing 24-OH levels in neurons to learn more about cholesterol control [36].

The link between blood and brain cholesterol levels may be explained by the oxysterols (oxidised forms of cholesterol) that are more abundant in hypercholesteremic diets [41,42]. The findings demonstrated that compared to the control group, The level of BDNF (brain-derived neurotrophic factor) was lower in those with hypercholesterolemia. Although between the cholesterol group and the control group, there was no statistically significant difference in terms of synapsin 1 levels, this finding is in conflict with [43,44,45,46] Cell survival depends on brain-derived neurotrophic factor, or BDNF, differentiation, maturation, learning, and memory, among many other brain activities. Many neurological illnesses have also been linked to abnormal BDNF expression. [47,48].

Hypercholesterolemia, in which elevated cholesterol levels cause an increase in the infusion of 27-OH into the brain, can cause neuronal damage in the neocortex; the amount of this damage may be dependent on the BDNF levels. Cholesterol is vital for cell maintenance, neural transmission, and synapse formation, and it is found in the brain (23% of total body cholesterol.[49]. Myelin sheath, which insulates axons, and dendritic spines are both heavily enriched with cholesterol. Studies have demonstrated that BDNF expression, neurogenesis, and lipid peroxidation are all negatively impacted by high-fat diets [50]. Specifically, oxidative stress and inflammation could influence BDNF levels [51].

ARC mRNA expression results

1-Examination of the gene expression using RT-qPCR data (Activity regulatory cytoskeleton (ARC) gene, Figure (4).

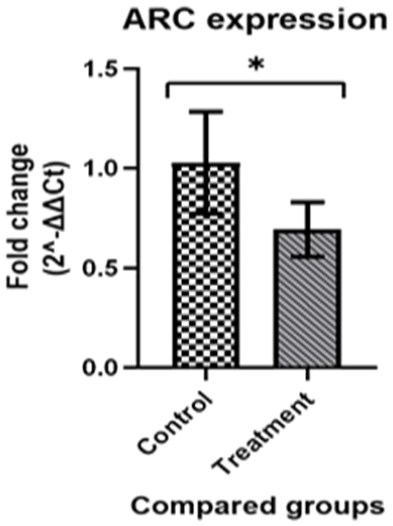


Figure (4): ARC mRNA expression in male rats brain tissue. The results show a significant decrease in ARC mRNA expression in the hypercholesteremic diet group compared with the control group($p<0.05$).

2- Efficiency of the assay's amplification (Activity regulatory cytoskeleton (ARC) gene, figure (5)A

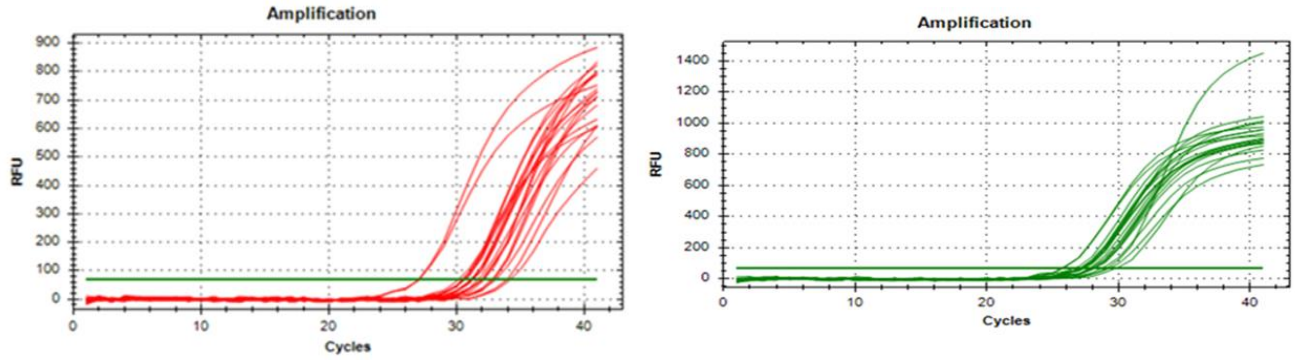


Figure (5): (A-B): A-amplification curve of the tested samples for expression of gene of interest (ARC). The successful amplification curves with the corresponding crossing threshold (CT) are the number of cycles with the round forming unit (RFU). The samples were tested in duplicates

B- amplification curve of the tested samples for the housekeeping gene (b-actin). As shown, the successful amplification curves have corresponding crossing thresholds (CT). The samples were tested in duplicates.

In response to intense synaptic activation, the mRNA and protein of the immediate early gene Arc (activity-regulated cytoskeletal-associated protein) are swiftly transported into dendrites and localised in locations of active synapses. Consequently, Arc mRNA and protein are put forward as a neuronal reactivity marker to trace the neural substrates recruited by different stimuli [52]. Moreover, there's mounting evidence that the immediate early gene Arc, also called Arg, plays a crucial role in activity-induced synaptic plasticity and certain types of behavioural memory [53,54].

The results of this investigation indicate that synaptic plasticity is accompanied with a decrease in Arc gene expression. animals that have high cholesterol Neuronal dendrites exhibit downregulation of Arc mRNA, where it binds to cytoskeletal proteins; furthermore, both its mRNA and protein are lost in dendrites in locations of recent synaptic activity, suggesting that Arc plays a unique function in synaptic plasticity [54 ,55 ,56].

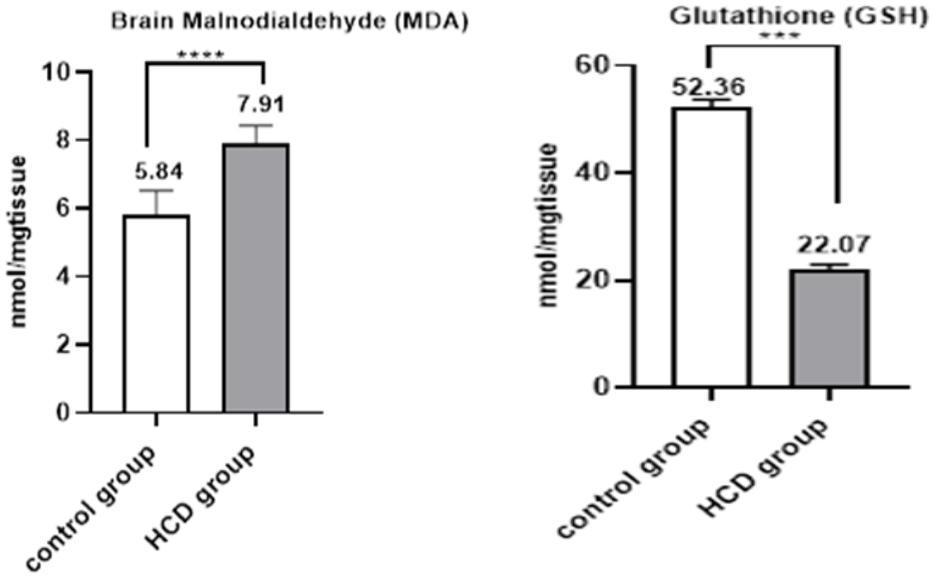


Figure (6): Effect of 1% hypercholesteremic diet for 4 weeks on the brain tissue Malnodialdehyde (MDA) and GSH concentration in the male rats.

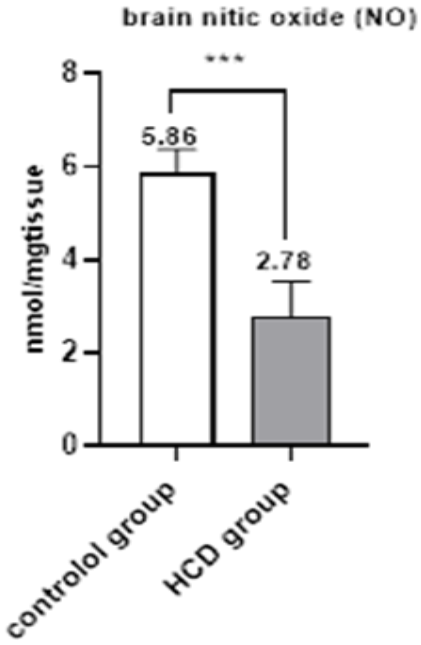


Figure (7): Effect of 1% hypercholesteremic diet for 4 weeks on the brain nitric oxide (NO) concentration in the male rats.

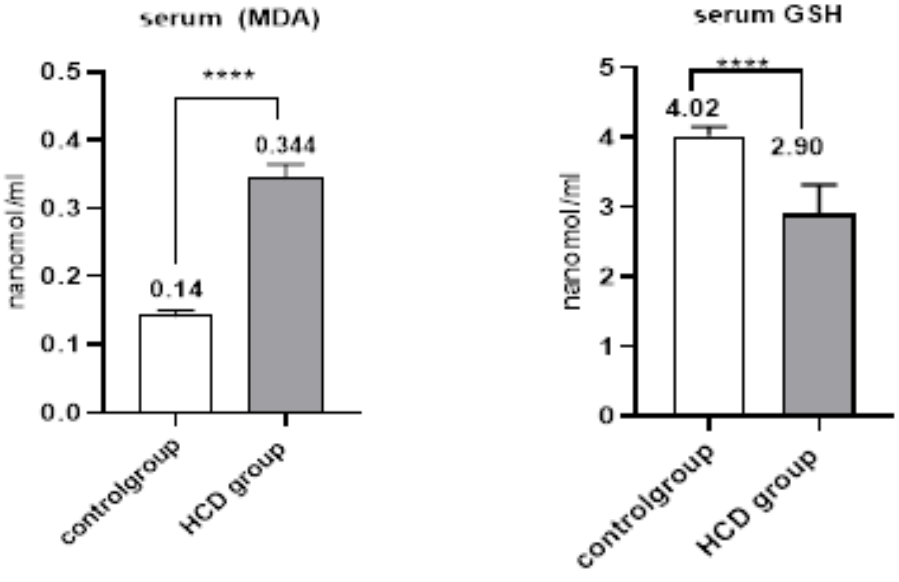


Figure (8): Effect of a 1% hypercholesteremic diet on serum MDA and GSH concentrations in male rats after four weeks of feeding

Cholesterol is a strong indicator of lipid peroxidation, a known mechanism of oxidative stress, as shown by a considerable decrease in brain and serum nitric oxide and glutathione (GSH) concentrations and a marked increase in malondialdehyde (MDA) levels relative to the control group. For this reason, anything that can reduce MDA levels is referred to as a free radical scavenger. came to an agreement on [57,58,59]. Cholesterol-mediated reduction of mitochondrial GSH is worsened by elevated cholesterol levels caused by poor mitochondrial GSH transfer [60, 61, 62]. This, in turn, alters mitochondrial function and increases ROS generation. By amplifying the mitochondrial oxidative damage induced by Aβ [9, 61]. Alzheimer's disease, which includes neuro-inflammation, is accelerated and made worse by brain cholesterol enrichment [63].

When molecular oxygen undergoes an incomplete reduction, it produces radicals and molecules known as reactive oxygen species. These species are essential for cellular homeostasis and play a critical function in communication [64].

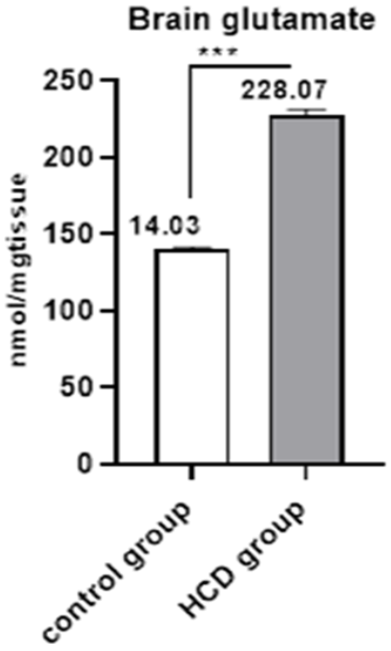


Figure (9): Effect of 1% hypercholesteremic diet for 4 weeks on the brain tissue glutamate concentration in the male rats

Brain interstitial fluid and blood are separated by the blood-brain barrier or endothelial cells, which are produced in animals in response to signals from brain cells. There are a number of reasons why the nervous system is physically separated from the circulatory system. One of these is that the amounts of glutamate in the blood are many times higher than what is harmful to neurons [65,66].

Several neurodegenerative disorders reduce the brain's cholesterol turnover. Impaired synaptic transmission may be linked to cognitive impairments and neurodegeneration.

Cholesterol biosynthesis defects can cause synaptic transmission failure, Sind Heimer's, Niemann-Pick type C, and Huntington's diseases[67].

Glutamate is the primary excitatory neurotransmitter in the healthy human brain because it is the most prevalent free amino acid and is found at the confluence of several metabolic pathways [67, 68,69].

Synaptic vesicles at nerve terminals store glutamate until its release, where it can rapidly build up in the extracellular fluid through exocytosis [69]. Also, outside the synaptic cleft, there are micromolar amounts of baseline extracellular glutamate that come from the cystine-glutamate antiporter's non-vesicular release [69,70,71].

Multiple neuroplasticity mechanisms, including glutamate's function as a neurostimulator, have been extensively studied and implicated in processes such as LTD, synaptic reorganisation, modulation of spine density, and long-term potentiation [72, 73]. Glutamate system dysfunction and decreased neuroplasticity can be consequences of persistent stress. Increased glutamate release, reduced LTP, apical dendritic atrophy, and learning and memory impairments are all consequences of chronic stress in the hippocampus [74].

Anxiety, impaired or enhanced LTP, dendritic hypertrophy, increased dendritic spines, and decreased glutamate release are all consequences of chronic stress, according to the current findings. An increase in glutamate and nitric oxide in brain tissue and serum reflects lipid peroxidation caused by free radical production. Most central nervous system processes rely on glutamate, and neuroplasticity—essential for adapting to environmental changes—also requires glutamate [68, 73,75].

Hypercholesteremia causes oxidative stress, which depletes BDNF and synapsin-1 and down-regulates ARC gene expression. This decreases glutamate and other master neurotransmitters in the brain, which affects learning and memory.

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