Effect of hypercholestermic diet on the amyloid precursor protein (APP) deposition with some behavior alterations in male rats

Hussein Adnan Raheem[1*], Wefak Albazi[1], Raeed Altaee [1], Goosoon Hadi Jhoni [2]

[1]Department of Physiology, College of Veterinary Medicine, University of Kerbala, Kerbala, Iraq.
[2]Department of Family Medicine, College of Medicine, University of Kerbala, Karbala, Iraq.
***Corresponding author e-mail: husseinalsead7750@gmail.com**
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Abstract

Hypercholesterolemic diet (HCD) is unusually high lipid levels in blood and is strongly linked to brain damage and cerebrovascular illnesses. HCD is a chronic brain disorder characterized by cognitive impairment, inflammation, β-amyloid(Aβ) deposition. The goal of this study to investigate behavioral changes caused by the HCD in male rats. The experiment employed sixteen male rats, which was randomly split into two groups, the control group (8/ group) and the treated group (8/ group), which received a 1% cholesterol daily for 28 days. Results of behavioral test showed a significant increase ($P \leq 0.0001$) in Morris water maze test (MWM) and Elevated plus-maze test /open arm in treated group compared to control group , while a significant decrease ($P \leq 0.0001$) in Elevated plus-maze test / closed arm treated group as compared to control group. Fold change comparison between the groups expressed amyloid beta precursor protein (App) gene, this shows significant downregulation of the treatment group compared with control group. We concluded that a HCD has negative behavioral alteration that are linked to gene expression changes.

Keywords: Hypercholesterolemia diet; behavioral alteration; gene expression (APP); Morris water maze (MWM); Elevated plus maze (EPM)

Introduction

Cholesterol is a waxy and fat-like substance with pivotal pathophysiological relevance [1] .A hydrophobic substance known as cholesterol is carried through the circulation by "lipoproteins" which are proteins , John Gofman used an ultracentrifuge to isolate the lipoproteins in plasma [1].

Multiple physiological conditions, such as fat, cardiovascular disease, and Alzheimer's disease, are brought on by high cholesterol levels [2]. Additionally, hypercholesterolemia, or elevated plasma cholesterol levels, are linked to male infertility because they cause the male reproductive system's malfunction [2]. A high-fat diet can worsen reactive stress and inflammation in the brain and has negative impacts on

cognitive function [3].An environment that is susceptible to harm from a high-fat diet is the aging brain [3]. cholesterol has been extensively implicated in the regulation of cellular amyloid precursor protein APP processing, contributing to the development of many disease specially in brain, the localization of (APP) in lipid rafts was increased by elevated cholesterol level[4,5,6].

The brain is particularly vulnerable to oxidative stress, which can be produced by an elevated cholesterol diet [7]. A high level of cholesterol and oxidative stress are substantial risk factors for a wide range of illnesses, including dementia and many central nervous system problems [7].

APP is a ubiquitously expressed protein that is cleaved to produce Apeptide fragments which can aggregate and lead to deposition of senile plaques, one of the pathological hallmarks of Alzheimer's disease (AD)[8,9].

Dysregulation of cholesterol homeostasis in the brain lead to considerably increase the risk of developing Alzheimer's disease (AD) and this dysregulation of lipid homeostasis may increase affecting APP cleavage, which is the most important risk factor involved in the pathogenesis of AD [9,10,11,12]. High cholesterol diet induced age-dependent effects on anxiety-like behavior and central neurochemical changes. High cholesterol diet might affect the central nervous system (CNS) function differently, and resulting in different behavior performance [13].

Hypercholesterolemia has been implicated as one of the most common neurodegenerative disorder affecting memory and learning abilities. By using Morris water maze(MWM) for knowing ameliorative effect of the memory and learning related behavioral performance of hypercholesterolemic . MWM developed by Richard, has become a popular tool for studying the neurobiology of hippocampus-dependent spatial learning and memory in rodents [14]**.** Hypercholesteremia on anxiety-like behavior tested in elevated plus maze and explored the relationship between cholesterol and anxiety-like behavior from the aspect of central neurochemical changes ,Elevated plus-maze test The elevated plus maze (EPM) is a widely used anxiety- like behavioral assay for rats [13,15].

The mechanism of behavioral changes as a result of the cholesterol metabolism disorder in the brain and related with the behavioral alteration is unclear thus the aim of the study is to investigate the effect of hypercholesteremia on the gene responsible for APP deposition and some behavioral tests [13,15].

Materials and Methods

Ethical approve

Under the reference number UOK.VET.PH.2022.046this study was conducted at the Kerbala University/ College of Veterinary Medicine's in Iraq's anatomical facility.

Experimental protocol

Sixteen white male albino rats weight (200g±20g) were used in this research and came from the College of Pharmacy at the University of Kerbala in Iraq. They ranged in age from (11–14) weeks, and the animals were housed in clean, specialized plastic

enclosures . We utilized a 12-hour light and a humidity of 55%. They were retained for two weeks so that they could adjust to the usual testing conditions . At the experiment's beginning 15th of December and ended at the 12th of January. Temperature was maintained at (23-26) C°.

Sixteen white male rats were arbitrarily split into two groups and given the following treatments for 28 days.

1- 8 rats made up of this group were given a regular meal orally as the comparison group.
2- 8 rats received cholesterol .Rats in this group were given a meal rich in cholesterol for 28 days, comprising 1% cholesterol pure powder (w/w) (8).

Determination of Morris water maze Test (MWM).

A spatial memory test was performed. The Morris water maze is a white circular pool (diameter: 150 cm and height: 35 cm) with a featureless inner surface circular pool was filled with nontoxic water and kept at 23-25 °C. The pool was divided into four equal-sized quadrants. A clear plastic platform (4.5) cm in diameter and(14.5) cm in height was placed in one of the pool's four quadrants. There are four significant visual signals on each side of the pool's four quadrants. The swimming path of rats from the starting point to the platform, which was submerged.

It was 0.5-1.0 cm below the water's surface, making it unnoticeable at water level. In the experiment, the rats were placed in the water labyrinth at one of three randomly chosen locations and then freed to discover the concealed platform. The experiment was terminated once the rats discovered and climbed onto the platform, and the escape latency was recorded. The maximum trial duration was 90 seconds. If animals did not locate the platform within 90 s, the experimenter guided the mouse by hand to the platform, then the rats was kept on the escape platform for 30 s and an escape latency of 90 s was recorded. order to assess the spatial retention of the location of the hidden platform, was conducted 24 h after the last acquisition session. During this trial, the platform was removed from the maze, and each mouse was allowed to search the pool for 90 s before being removed. The time spent in the target quadrant was used as a measure of consolidated spatial memory [16].

Determination of Elevated Plus-Maze Test (EPM-T):

Elevated plus maze from rats were tested on the elevated plus maze . The polypropylene maze was elevated 1 M from the ground with four 10.cm wide×50.cm long runways . The two closed runways had 50 cm polypropylene walls. To begin the test, rats were placed at the center of maze facing a closed arm. Plus-maze behavior was measured as the time spent of the maze during a 10 min. session as determined by the average of counts recorded by an automated system and by an observer blind to the experimental treatment condition. For subjective rating, entries were recorded when a rat had four paws on an arm and explorations were recorded when a rat had two paws on an arm. Time spent on the open arms was defined as the sum of the intervals between open and closed arm entries [15,17].

Collecting of organs for gene expression Amyloid precursor protein (APP):

Samples were collected after the animals were killed. The brain tissue was taken placed in liquid nitrogen in order to be preserved and transported to the laboratory for extraction and examination.

Analytical statistics

The statistical program Graph Pad Prism 8.0 the t-test was used, P≤0.05 was chosen as the standard of significance. The data points were shown as mean± SD.

Primers used in this study

Table (1): Primers of gene expression experiment

Primer name	Sequence '5---------3'	Target gene
APP F	TGATGTCACTGAAGGAAAGTGC	App
APP R	GAGGTTCACTGGTAGTCTTGAGTA	
Rat-GAPDH-F	GTGGACCTCATGGCCTACAT	Internal reference gene (normaliser)
Rat-GAPDH-R	GGATGGAATTGTGAGGGAGA	

Results and Discussion

Effect cholesterol on Morris water maze test (MWM)

Cholesterol is a widely recognized important lipid membrane modulator structure and fluidity, and as such, it is crucial for maintaining transmembrane transmission both within and between cellular divisions. Cholesterol amounts in membranes are necessary for living. Despite making up only 2.2% of the total body weight, the central nervous system (CNS) includes up to 25% of the body's overall cholesterol[18,19,20].

The level of Morris water maze test (MWM) were measured following 28 days experiments show that there was a significant increase (P≤0.0001) in treated group (82.13+2.49) as compared to control group (45.38 ± 2.494) show in figure(1).

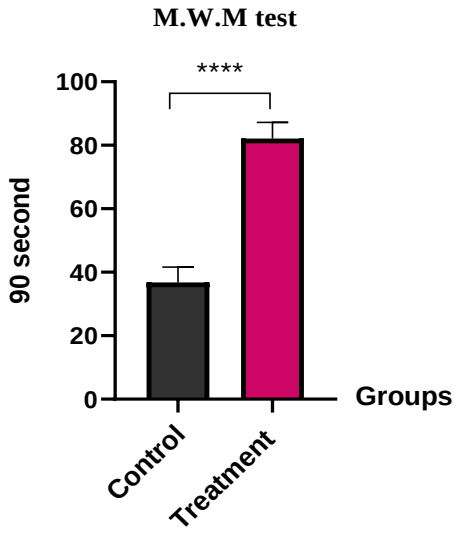


Figure (1): Effect cholesterol on Morris water maze test (MWM) in the brain damage of male rats.

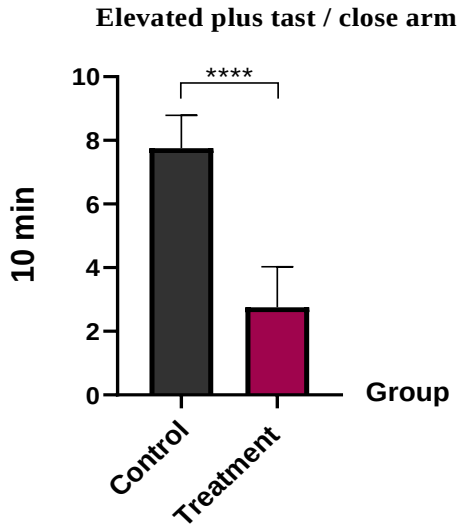
When treatment groups were compared to control groups, there was a significant difference in the time of the Morris water maze test (MWM) (****P < 0.0001).
A few investigations have looked at the relationship between serum cholesterol levels and animal behavior. In this work, we looked at the impact of high cholesterol diets on rat behavior, as well as the probable link between cholesterol and behavior from the standpoint of central neurochemical alterations ,this result is agreement with [14,21,22].

Memory experiments utilizing MWM are based on the idea that the experimental animals will want to get out of the water and find the submerged platform as quickly as feasible. The animals' memorising capacity increases as the seeking duration shortens. Similarly, rats with superior memory would cover less distance to reach the platform, but rats with weak memory would be perplexed and circumlocute for a considerable distance to find the platform [14,23].

In the current MWM studies, the hypercholesterolemic rats suffered from impaired escape latency and distance swimming performance, which indicated memory and learning related behavioral alteration [14] .

Effect cholesterol on Elevated plus-maze test / closed arm

The level elevated plus-maze test / closed arm there was a significant decrease (P≤0.0001) treated group(2.750±1.28) as compared to control group (7.750±1.03) showed in figure (2).

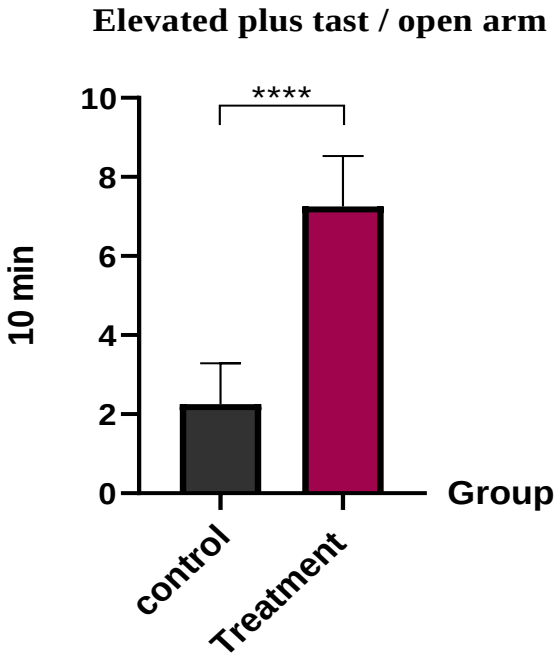


Figure(2): Effect cholesterol on Elevated plus-maze test / closed arm on brain damage of male rats.

After comparing the treatment group to the control group, there was a substantial decrease in the time of the Elevated plus-maze test closed arm (****P < 0.0001).

Effect cholesterol on Elevated plus-maze test / open arm

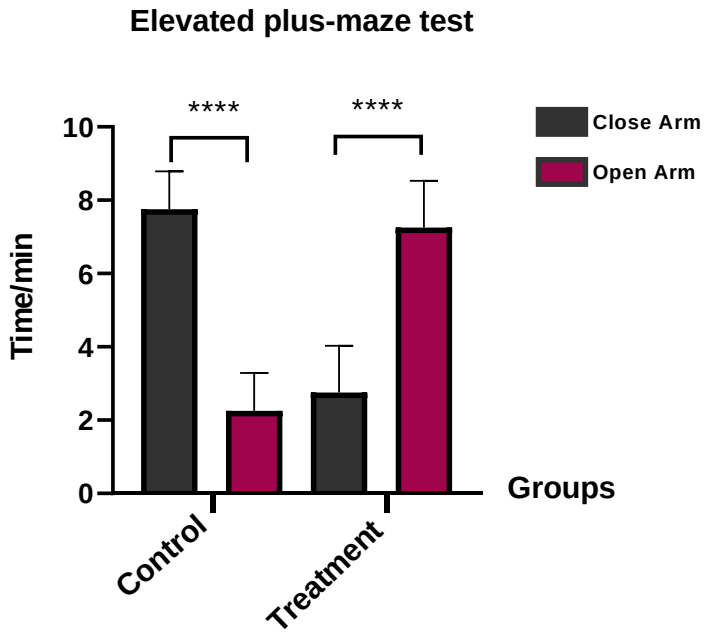
A significant increase (P < 0.0001) in The level elevated plus-maze test / open arm in the treated group (7.250±1.28) compare with control group (2.250±1.03) figure (3).



Figure(3): Effect cholesterol on Elevated plus-maze test / open arm on brain damage of male rats.

There was a significant increase in the time of Elevated plus-maze test \ closed arm in group treatment compare to control group. (****P < 0.0001).

A significant difference in the time of Elevated plus-maze test / open arm and closed arm in treatment group compare to control group. (P < 0.0001) was show in Figure (4)



Figure(4): Effect cholesterol on Elevated plus-maze test / open & closed arm on brain damage of male rats.

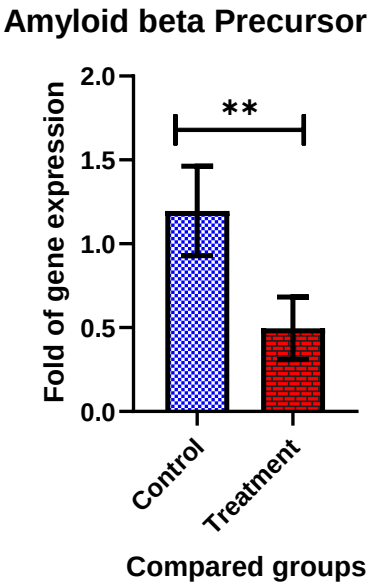
There was a significant difference in the time of Elevated plus-maze test / open arm and closed arm in treatment group compare to control group. (****P < 0.0001).

In test of the Elevated plus-maze/open &close arm , the treated group spent much more time with open arms and significantly less time with closed arms than the control group this result is agreement with [13] .

High cholesterol diet might result in an increase of brain cholesterol content. cholesterol might be beneficial for the growth and differentiation of the CNS. Therefore, HCD in rats, the growth and differentiation of CNS is mature, and induced excessive accumulation of cholesterol might affect brain function [13].

Expressed amyloid beta precursor protein (App) gene

Fold change comparison between the groups expressed amyloid beta precursor protein (App) gene. This shows significant downregulation of the treatment group compared with control group see in figure (5).



Figure(5): Fold change comparison between the groups expressed amyloid beta precursor protein (App) gene.

This shows significant downregulation of the treatment group compared with control.

Result Gene expression of amyloid beta precursor protein (App):

1- **Analysis of the RT-qPCR gene expression data (**amyloid beta precursor protein (App) gene**):**
2- **Efficiency of the assay's amplification (**amyloid beta precursor protein (App) gene**) .**Figure(6): amplification curve of the tested samples represents the amyloid beta precursor protein (App) gene. This indicates a successful RNA extraction and cDNA synthesis

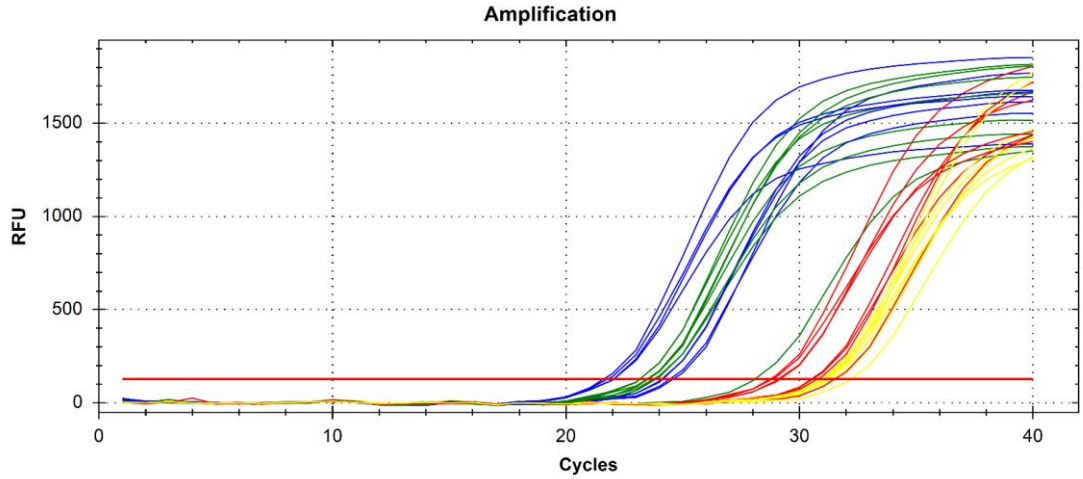


Figure (6): amplification curve of the tested samples represents the amyloid beta precursor protein (App) gene. This indicates a successful RNA extraction and cDNA synthesis.

Note:
Yellow curves = APA treatment group
Red curves = APA control group
Blue curves = Housekeeping control group
Green curves = Housekeeping treatment group

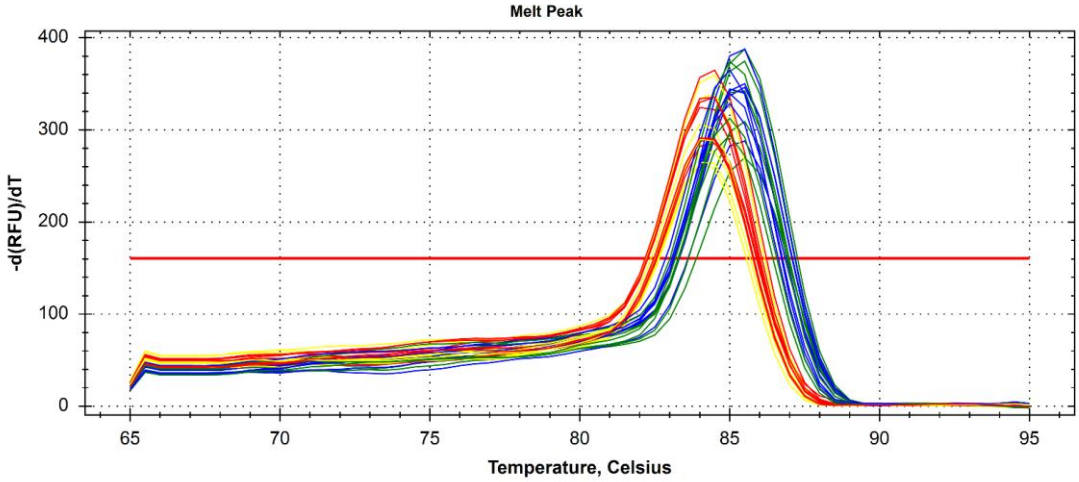


Figure (7): Disassociation curves analysis of the amplified products of amyloid beta precursor protein (App) gene (gene of interest).

This shows a high specific amplification without non-specific reaction or primer dimer.

Note:
Yellow curves = APA treatment group
Red curves = APA control group
Blue curves = Housekeeping control group
Green curves = Housekeeping treatment group

A fragment of APP, is the major peptide of senile plaques.44 Until now, the upstream factors leading to the formation of tangles and plaques was not fully understood [24]

Different tests with protocols have been developed to assess spontaneous alternation behavior including MWT, rats suffering from APP deposition were previously tested at MWM test where poor response times appear indicating cognitive impairment, making it a relevant task for studying the role of APP. [9,25,26].

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