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ORIGINAL ARTICLE

The Possible Protective Effects of Chrysin on Cerebral and Cerebellar Cortices of Adult Male Albino Rats Exposed to Induced Hyperlipidemia (Histological and Immunohistochemical Study)

Samar Abdelaziz Mostafa[1]*, Samar Mohamed Reda[1], Reham I. Elgarhi[2], Karima F. Abdelfadeel[1]
[1]Medical Histology Department, Faculty of Medicine, Zagazig University, Egypt
[2]Pharmacology Department, Faculty of Medicine, Zagazig University, Egypt
***The corresponding author**
Samar Mohamed Reda

Email:
drsamrreda@yahoo.com

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Abstract

Background: Hyperlipidemia is an important public health problem. It has been linked to numerous disorders of the neurological system. A well-known flavonoid with a variety of medicinal uses is chyrsin. Therefore, the current study objectives were to identify the effects of high fat diet (HFD)-induced hyperlipidemia on the cerebral and cerebellar cortex of adult male albino rats, and to assess the potential protective effect of chrysin. **Methods:** Forty five rats were divided into three groups: Control group, Hyperlipidemic group which were fed HFD for four weeks, and Hyperlipidemic+ Chrysin group which were fed HFD+ Chrysin. **Results:** Hyperlipidemia caused significant increase in serum cholesterol, LDL and TG, while serum HDL was significantly-decreased. There were significant increase in brain levels of TNFα, IL6, IL1, amyloid beta (Aβ), VEGF and MDA, and significant decrease in brain GSH and SOD with histological alterations in sections of the cerebrum and cerebellum. Some neurons appeared with apoptotic nuclei which characterized by neuronal shrinkage and chromatin condensation and congested blood vessels between them. Also, weak stained Nissel's granules in the cytoplasm of neurons and cytoplasm of Purkinje cells were noticed. Hyperlipidemic group showed significant increase in area percent of GFAP and optical density of caspase 3 immunoreactions. **Conclusions:** Usage of chrysin ameliorated both the biochemical and the morphological changes induced by HFD administration. Chrysin is able to protect against neuronal cell damage by inhibiting oxidative stress and inflammation.

Keywords: Cerebral cortex; Cerebellar cortex; High fat diet; Hyperlipidemia; Chrysin.

INTRODUCTION

Globally, the incidence and prevalence of hyperlipidemia are rising, making it a serious public health concern. The hyperlipidemia is caused by the modern lifestyle, which includes a diet heavy in fat and little activity, is considerably exacerbated[1].

Elevated serum total cholesterol (TC), low-density lipoprotein cholesterol (LDLC), triglyceride levels,and/or decreased levels of highdensity lipoprotein cholesterol (HDLC) are the hallmarks of Hyperlipidemia.[2].numerous studies have shown That hyperlipidemia is a substantial risk factor for chronicrenal impairment, fatty liver disease,atherosclerosis,and cardiocerebrovascular disease. Other studies have also demonstrated that neuroinflammation brought on by hyperlipidemia leads to neuronl Injury [3].

Pierrot and Octave, [4] mentioned that as a significant risk factor, hyperlipidemia has been linked to numerous disorders of the neurological system. For instance, Niemann-Pick disease and Alzheimer's disease are both linked to hyperlipidemia. Paul et al. [5] also revealed that in a mouse model of Parkinson's disease,

hypercholesterolemia accelerated dopaminergic neurodegeneration in the midbrain. An independent dementia risk factor is hyperlipidemia. High-fat diet- (HFD)-induced obesity increases the incidence of type 2 diabetes and impairs brain processes [6].

It is widely acknowledged that a high-fat diet is one of the major factors contributing to global obesity and hyperlipidemia [7]. Furthermore, one ongoing problem in patients with hyperlipidemia is the increased generation of free radicals and reactive oxygen species (ROS) [8].

Although many synthetic lipid-lowering medications including fibrates, statins, and bile acid sequestrates, have been created to treat hyperlipidemia, management of this condition without accompanying drug side effects still poses a challenge [9]. Hyperuricemia, diarrhoea, nausea, myositis, stomach irritation, flushing, dry skin, altered liver and renal function are all side effects of synthetic drug use [10]. Over the past ten years, there has been an increase in the usage of herbal medicine and medicinal plants all over the world. Patients are encouraged to use medicinal plants as the first line of treatment because they are risk-free for health and have no negative effects [11].

Flavonoids are abundant in many fruits and veg etables and are used successully to treat a variety o f medical diseases due to their various pharmacolo gical properties. They also show tremendous promi se for further research. Flavonoids have a variety o f biological roles as well as pharmacological action s, such as antiinflammatory, anticancer, antibacteri al, and antioxidative properties [12].

A well-known flavonoid called chysin (5,7-dihydroxyflavone) is present in propolis, honey, Indian trumpet flower (Oroxylum indicum) and blue passion flower (Passiflora caerulea) [13]. Due to its antioxidant and anti-inflammatory qualities, it has drawn attention. According to several research, chrysin reduces inflammation by preventing the synthesis of cytokines that cause inflammation. The ability of chrysin to neutralise free radicals and guard against oxidative stress has also been demonstrated [14].

Chrysin has a variety of medicinal benefits, including anti-diabetic, anti-cancer, nephroprotective, cardioprotective, anti-arthritic, and antiasthmatic qualities [15]. As a result, the current study goals were to determine the effects of hyperlipidemia on the cerebrum and cerebellum of adult male albino rats, and to evaluate any potential protective effects of chrysin using biochemical, histological, and immunohistochemically methods.

METHODS

Drugs and Chemicals:

Chrysin, 5,7-Dihydroxyflavone, 98% was purchased from SIGMA Pharmaceutical Company, Egypt, in the form of white crystalline powder.

Experimental animals:

For this work, 45 adult male albino wistar rats were used. They were 10 to 18 weeks old, and weighed 180 to 200 grams. The animals were obtained from the Zagazig University Faculty of Medicine Breading Animal House. They were kept in a room at a constant temperature with regular light and dark cycles and unrestricted access to food and water. Before beginning the experiment, they spent two weeks becoming acclimated to their surroundings. The Faculty of Medicine at Zagazig University complied with and approved the Institutional Animal Care and Use Committee's recommendations (Approval number: ZU-IACUC/3/F/157/2023).

Induction of Hyperlipidemia:

For four weeks, the animals were given a high-fat diet (HFD) that contained 6.3 kcal/g of energy; 19% from protein, 35% from fat, and 46% from sugar. With free access to HFD, the diet consists of a mixture of 69% regular chow pellets, 6% corn oil, 19% milk powder and 6% ghee. Dietary composition (g/kg diet) was calculated using the Fahmy et al. formula [16]. The HFD ingredients were bought from El-Gomhoria Company in Cairo, Egypt, and stored at 4°C until they were needed.

Experimental design:

The rats were randomly divided into three groups:

Group I (Control): including 27 rats that were fed the standard rodent chow for four weeks with free access to diet. The standard rodent chow diet contains 3.16 kcal/g with 21% from protein, 48.8% from sugar and 3% from fat. They were divided into three equal subgroups, each included 9 rats.

Subgroup Ia: rats were left without intervention to measure the basic parameters

Subgroup Ib (Vehicle of Chrysin): in which 1ml of distilled water was given daily by oral gavage for four weeks.

Subgroup1c (Chrysin): rats received Chrysin every day by oral gavage at a dose of 50mg/kg body weight dissolved in 10 ml distilled water daily for four weeks [17].

Group II (Hyperlipidemic group): including 9 rats that were fed a high-fat diet (HFD) for four weeks with free access to diet.

Group III (Hyperlipidemic+Chrysin): including 9 rats that were fed a high-fat diet (HFD)+Chrysin at a dose of 50mg/kg body weight dissolved in 10 ml distilled water daily for four weeks.

General observation:

Throughout the experiment, the general appearance and daily food consumption of the rats were observed; rats mortalities and weight were recorded also.

Biochemical analysis:

Blood samples:

Once the experimental time is over, by using a heparinized capillary tube, blood samples were separately taken from each rat eye and transferred into sealed tubes without an anticoagulant. Using a centrifuge, the blood was centrifuged at 2500 rpm for 20 minutes to extract the serum for biochemical analysis after the blood had been allowed to clot at room temperature for 30 minutes. Supernatant of the yellow serum was eliminated. Serum triglycerides, total cholesterol, low density lipoproteins (LDL), and high density lipoproteins (HDL) concentrations were all measured using the spectrophotometer analyzer.

Methods: Serum cholesterol, triglyceride: ,high density protein (HDP), low density protein (LDP): were measured according to method explained by the following references [18,19,20, &21] respectively.

Tissue samples:

Specimens from the cerebral and cerebellar cortices were collected by the end of the experiment; they were homogenized in prepared basic solvent (phosphate buffered saline for ELISA measures). Other specimens were homogenized in menthol for cholesterol and TG measure. Then, samples were centrifuged for one hour to take the membranes.

The levels of brain cholesterol and triglycerides were detected using enzymatic methods of glycerol phosphate oxidase-peroxidase-4-aminoantipyrine (GPO- PAP)

IL-1 (ABclonal cat n. RK00009), IL-6 (ABclonal cat n.RK00020), TNF- α (Mybiosource cat n. mbs2507393), VEGF (ABclonal cat n. RK00066) and amyloid beta (Aβ) (LSBio cat n. LS-F23254) were measured in brain specimens using a biotin- avidin-based ELISA kits.

Brain tissue MDA (nmol/g tissue) was measured by using kits (ABclonal cat n.RK09070) as described by Raquel et al. [22]. Brain tissue SOD (U/g tissue) was measured by using kits (ABclonal cat n. RK03959) as described by Nishikimi et al. [23]. Brain tissue CAT (U/g tissue) was determined by using kits (ABclonal cat n.RK03551) as described by Aebi [24], and brain tissue GSH level (mg/g tissue) was determined by using kits (ABclonal RK15286) as described by Beutler et al. [25].

Histological study:

Rats from all groups were sacrificed at the end of the experiment using an intraperitoneal injection of 50 mg/kg sodium thiopental [26]. The cerebrum and cerebellum samples were taken shortly after sacrifice. For light microscopic examination, sagittal cerebral and cerebellar slices were fixed in 10% neutral formol saline, dried, embedded in paraffin wax, and cut into 5 µm sections [27]. They were stained with:

Hematoxylin and eosin: for routine histological examination [27].

Cresyl violet: for detection of Nissel's granules [27].

Immunohistochemical examination:

Immunostaining was done using the streptavidin biotin-peroxidase technique according to Ramos-Vara et al [28] for recognition of:

a. Glial fibrillar acid protein (GFAP): Using a mouse Monoclonal Anti-Glial Fibrillary Acidic primary antibody of IgG type (clone G-A-5, product No.P14136) with a minimum working dilution of 1:400. It was obtained from Sigma-Aldrich, Steinheim, Germany.

b. Caspase-3: Using a rabbit anti caspase 3 monoclonal primary antibody of IgG fraction (CAT. Number MAB4703) with a minimum working dilution of 1:500. It was obtained from Sigma-Aldrich, Steinheim, Germany.

Examination and photography:

Examination and photography of the sections were done at the Department of Medical Histology and Cell Biology, Faculty of Medicine, Zagazig University.

Morphometric study:

H&E- stained sections from the cerebellum of different studied groups were morphometrically analayzed for the mean area percentage of Purkinji layer thickness. Also, immunohistochemical-stained sections from the cerebral and cerebellar cortices of different studied groups were

morphometrically evaluated for the mean area percentage of GFAP immunoexpression, and the optical density of caspase-3 immunereaction by Fiji image J (1.51n, NIH, USA) program at the image analysis unit, Department of Pathology, Faculty of Dentistry, Cairo University. The image analyzer consisted of a colored video camera, colored monitor, and an IBM hard disc that was linked to the microscope and controlled by Leica Qwin 500 software. Calibration of the image analyzer was done first automatically to obtain the actual micrometer units from the measurement units (pixels) generated by the image analyzer program. A system of computer image analyzer was used. For each parameter, 10 non overlapping histological fields (all fields have the same diameters) were selected from each slide, using an objective lens of magnification x 40. The mean values for each parameter in the different groups were determined.

Statistical analysis:

Statistical Package for the Social Sciences (SPSS) version 20.0 software was used to analyze the data collected for body weight, food consumption, biochemical analysis, and morphometrical analysis. The data were expressed as mean SD (standard deviation) and subjected to one-way analysis of variance (ANOVA) for differences between the mean of different groups. We conducted additional analysis utilizing the post hoc test to compare the parameters of the various groups with one another. For two-tailed tests, a probability of p value less than 0.05 was regarded as statistically significant, and a result of 0.001 seemed very significant.

RESULTS

General observation: The rats behaved normally throughout the experiment as regard drinking water, eating food and defecating, and behaviors were consistent. No mortalities were detected. In comparison with the control group, the hyperlipidemic and the hyperlipidemic+chrysin groups showed non-significant difference regarding to the food consumption. Regarding the body weight, the hyperlipidemic group showed statistically significant increase in comparison to the control group. However, there was no statistically significant difference between the control group and the hyperlipidemic+ chrysin group **(Table S1).**

Biochemical results:

Biochemical analysis of subgroups Ia, Ib, Ic revealed closely similar results. Therefore, the results of subgroup1a were only reported.

a. Effect of chrysin on Serum level of cholesterol, TG, LDL and HDL presented as mean±SD (Table S2):

The results showed that there is significant increase in cholesterol, TG, LDL levels in hyperlipidemic group compared to control group, while there is no significant difference between Hyperlipidemic+chrysin treated group and control group. There is significant decrease in HDL level between hyperlipidemic group and control group but there is no significant difference between Hyperlipidemic+chrysin treated group and control group

b. Effect of chrysin on cerebral level of cholesterol and TG presented as mean±SD (Table S3):

The results showed that there is significant increase in cerebral level of cholesterol and TG in hyperlipidemic group compared to control group while there is no significant diffrence between Hyperlipidemic +chrysin treated group and control group.

c. Effect of chrysin on cerebral level of TNFα, IL6, IL1 presented as mean±SD (Table S3):

The results showed significant increase in TNFα, IL6 and IL1 in hyperlipidemic group compared to control group and no significant difference between Hyperlipidemic+chrysin treated group and control group.

d. Effect of chrysin on cerebral level of amyloid beta (Aβ) and VEGF presented as mean±SD (Table S3):

The results showed significant increase in amyloid beta and VEGF levels in hyperlipdemic group compared to control group, while there is no significant difference between Hyperlipidemic+chrysin treated group and control group

e. Effect of chrysin on cerebral level of MDA, GSH, SOD presented as mean±SD (Table S3):

The results showed significant increase in MDA level, significant decrease in GSH and SOD in hyperlipidemic group compared to control group, while there is no significant difference between Hyperlipidemic+chrysin treated group and control group. **Morphometric and statistical results:**

Morphometric and statistical results of subgroups Ia, Ib, Ic revealed closely similar results.

Therefore, the results of subgroup1a were only reported.

a- Mean values (± SD) of morphometric parameters of the cerebral cortex in the studied groups (Table S4):

The results showed significant statistical difference among the studied immunoexpression of GFAP mean area percent immunoexpression in the cerebral cortex with the lowest level was the control group and the greatest level was found in the hyperlipidemic group. There was significant difference between the hyperlipidemic group and both control group and hyperlipidemic+ chrysin group while no significant difference between control group and hyperlipidemic+chyrsin group. Significant statistical difference between the examined groups concerning the optical density of caspase3 with the greatest level was found in the hyperlipidemic group. No Significant difference between control group and hyperlipidemic+ chrysin group was recorded.

b- Mean values (± SD) of morphometric parameters of the cerebellum in the studied groups (Table S5):

The results showed significant statistical difference among the studied immunoexpression GFAP mean area percent immunoexpression in the cerebellum with the lowest level was the control group and the greatest level was the hyperlipidemic group. There was significant difference between the hyperlipidemic group and both control group and hyperlipidemic+ chrysin group while no significant difference between control group and hyperlipidemic + chrysin group.

Histological results:

All control subgroups (a, b and c) were examined by the light microscope, and revealed closely similar results. Therefore, the histological results of subgroup Ia only were recorded.

Examination of H& E-stained sections of the cerebral cortex revealed the control group neurons with basophilic cytoplasm and large vesicular nuclei. The glial cells with small dense nuclei were also seen. The blood vessels appeared normal. The group exposed to hyperlipidemia appeared with neurons containing vesicular nuclei and others showed chromatin condensation. Congested blood vessels were seen. The hyperlipidemic+ chrysin group displayed neurons with vesicular nuclei surrounded by glial cells and mild congested blood vessels **(Figure 1).**

H& E sections of the cerebellar cortex of the control group showed the three layers of the cerebellar cortex; the outer molecular layer, the middle Purkinje cell layer and the inner granular layer. The Purkinje layer appeared with vesicular nuclei, the granular layer contained numerous small, densely packed granule cells with spherical, highly pigmented nuclei and sparse cytoplasm. There were many lightly pigmented acidophilic regions that resemble glomeruli. The group exposed to hyperlipidemia revealed some cells arranged in single layers and others appeared in double layers of Purkinje cells. Cells have darkly pigmented nuclei and appeared shrunken. The granular cells appeared clumped, darkly stained and shrunken. The hyperlipidemic+ chrysin group showed some normal Purkinje cells with a vesicular nuclei and others total degenerated with ghost shape appearance. The granular and the molecular layers appear nearly normal (**Figure 2).**

The control group's cerebral cortex cresyl violet stained sections examination revealed neurons with darkly stained Nissel's granules surrounding a large negative stained nuclei. The cytoplasm of the neurons of the hyperlipidemic group, Nissel's granules were weakly stained.The cells of the hyperlipidemic+ chrysingroup displayed larger neurons, weakly stainedcytoplasm, and Nissel's granules around a darkly stained nuclei **(Figure 3).**

The cresyl violet sections of the cerebellum tissue of the control group showed darkly stained Nissel's granules around a large negative stained nucleus. The group exposed to hyperlipidemia showed weak stained Nissel'sgranules in the cytoplasm of Purkinje cells. The hyperlipidemic + chrysin group appeared with some Purkinje cells with darkly stained Nissel's granules surrounding a large nucleus and others with weak stained granules **(Figure 4).**

The sections of immunoreaction with Caspase 3 in the cerebral cortex showed negative nuclear reaction to the Caspase–3 antibody. Few cells appeared with positive reaction on the control group. The group exposed to hyperlipidemia showed most of neurons with strong positive nuclear reaction to the Caspase–3 antibody. The hyperlipidemic+ chrysin group sections appeared with positive nuclear reaction to the Caspase–3 antibody in some neurons. The sections of immunoreaction with GFAP in the cerebral cortex showed weak cytoplasmic reaction in the glial cells with star-shaped appearance, slim bodies and thin

long processes in the control group. The group exposed to hyperlipidemia showed strong cytoplasmic reaction in the glial cells with hypertrophied cell bodies, increased number and thickness of dendritic processes so increased GFAP immunoreactivity was observed. The Hyperlipidemic+ chrysin group showed moderate cytoplasmic reaction in the glial cells with less cell body hypertrophy and thin processes **(Figure 5).** Examination of sections of immunoreaction with Caspase 3 in the cerebellum from the control group showed negative reaction to caspase–3 antibody. Few cells appeared with faint nuclear reaction. The group exposed to hyperlipidemia showed most of neurons with strong positive nuclear reaction to the Caspase–3 antibody. The Hyperlipidemic+ chrysin group showed some neurons with positive nuclear reaction to the Caspase–3 antibody. Examination of the sections with GFAP immunoreaction in the cerebellum revealed moderate cytoplasmic reaction in the glial cells which had thin processes in the control group. The group exposed to hyperlipidemia showed strong cytoplasmic reaction in the the molecular layer, Bergmann-glia (modified astrocytes) were perpendicular to the surface and parallel to each other but in the granular layer, true astrocytes appeared star-shaped. The hyperlipidemic+ chrysin group showed moderate cytoplasmic reaction in the glial cells. Bergmann- glia and true astrocytes were noticed **(Figure 6).**

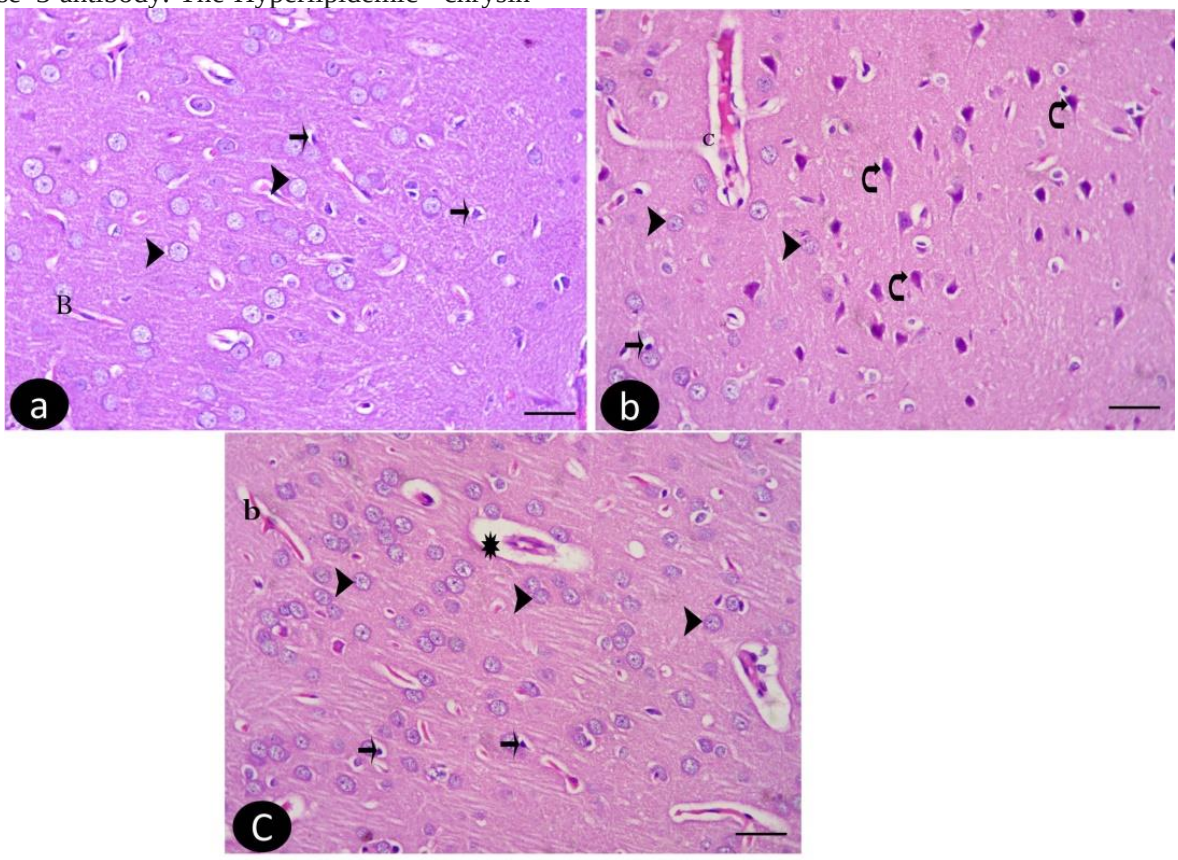

Figure (1): Photomicrographs of H&E sections in the cerebral cortex **a-** control group showing neurons with basophilic cytoplasm and vesicular nucleus (arrow head). Glial cells with small dense nuclei (arrow) and normal blood vessels (B) are also seen **b-** The group exposed to hyperlipidemia showing neurons with vesicular nuclei (arrow head) and others with apoptotic nuclei which are characterized by neuronal shrinkage and chromatin condensation (curved arrow). A congested blood vessel can be seen (c). **C-**The hyperlipidemic+chyrsin group showing neurons with vesicular nuclei (arrow head) surrounded by glial cells (arrow). Some blood vessels reveal mild congestion (star) while others appear normal (b). **(H and E, X400, scale bar 20 um).**

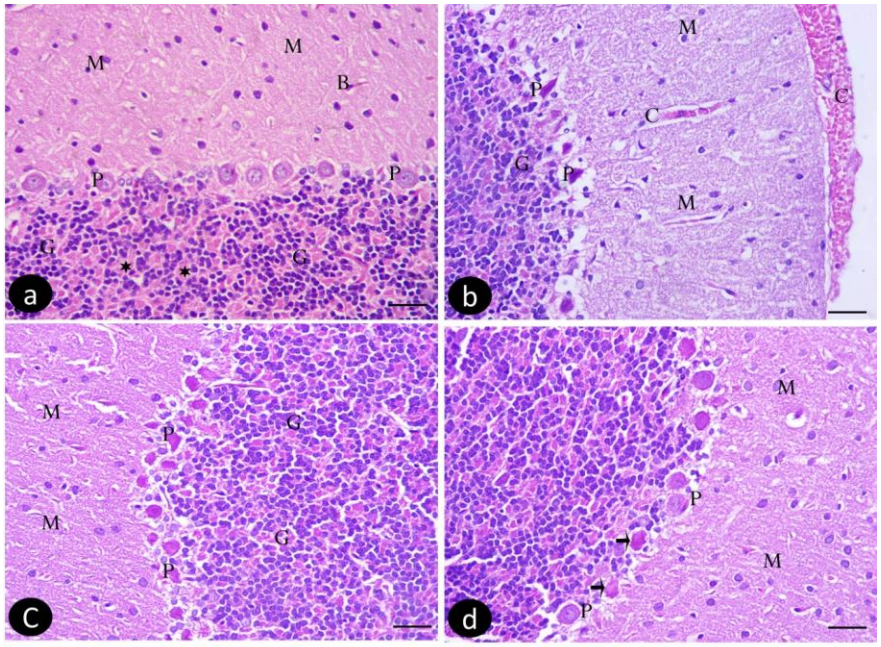

Figure (2): Photomicrographs of H&E sections in the cerebellum **a-** control group showing the three layers of the cerebellar cortex; the central Purkinje cell layer with large cells and vesicular nuclei (P), the inner granular layer (G), and the outer molecular layer (M). The granular layer is made up mostly of numerous small, densely-packed granule cells with spherical, highly pigmented nuclei and sparse cytoplasm. There are many lightly pigmented acidophilic regions that resemble glomeruli (star). A normal blood capillary is noticed (B). **b-** The group exposed to hyperlipidemia showing Purkinje cells with shrunken darkly pigmented pyknotic nuclei (P). Molecular layer (M) appears with congested blood vessels (B). Granular layer (G) is noticed. **C-** The cerebellar cortex from rats exposed to hyperlipidemia also shows double layers of Purkinje cells which appear shrunken with pyknotic nuclei (P). Molecular (M) and granular layers (G) are also seen. **d-** The hyperlipidemic+chyrsin group showing some normal Purkinje cells with pale nuclei (P). Other cells are degenerated with ghost appearance (arrow). The granular (G) and the molecular (M) layers look nearly similar to the control. **(H and E, X400, scale bar 20 um).**

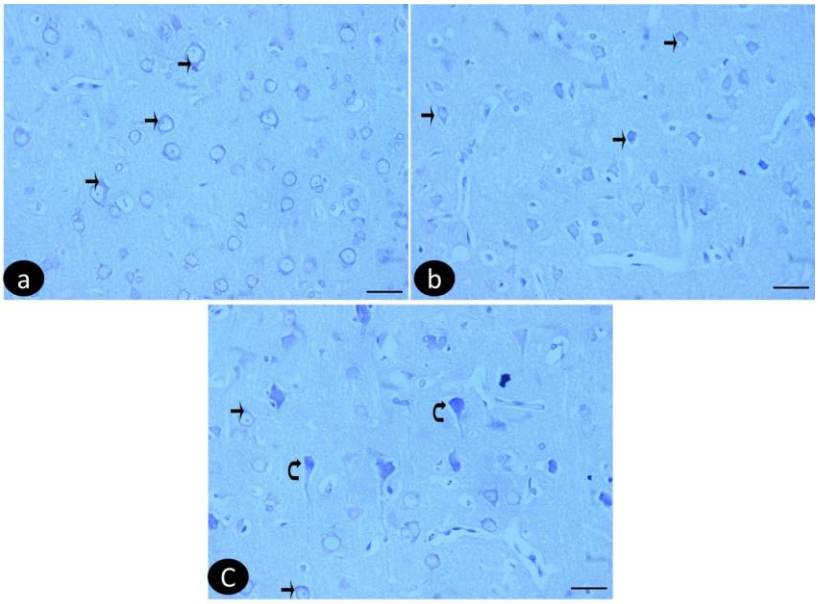

Figure (3): Photomicrographs of cresyl violet sections in the cerebral cortex: **a-** The control group showing neurons with darkly-stained Nissel's granules (arrow) around large negative stained nuclei. **b-** The group exposed to hyperlipidemia showing Nissel's granules (arrows) around irregular shaped nuclei. **C-** The hyperlipidemic+chyrsin group showing neurons with darkly stained Nissel's granules (arrow), together with irregular dark neurons (curved arrow), **(Cresyl violet X400, scale bar 20 um).**

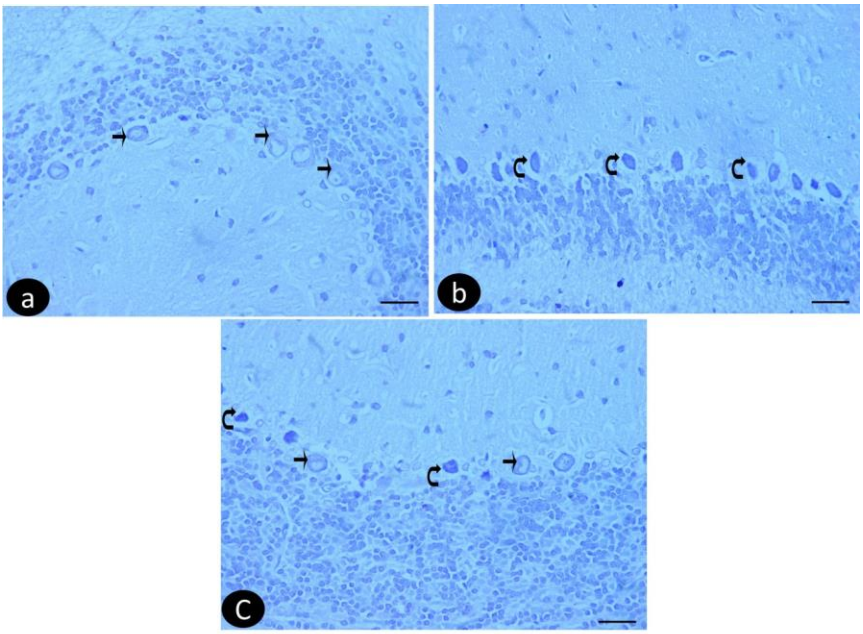

Figure (4): Photomicrographs of cresyl violet sections in the cerebellum: **a-**The control group showing darkly stained Nissel's granules (arrow) around large negative stained nuclei. **b-** The group exposed to hyperlipidemia showing Nissel's granules (arrows) in the cytoplasm of Purkinje cells around irregular nuclei. **C-** The hyperlipidemic +chyrsin group showing some Purkinje cells with dark stained Nissel's granules (arrow) surrounding large vesicular nuclei, and other granules (curved arrow) are surrounding irregular dark nuclei **(Cresyl violet X400, scale bar 20 um).**

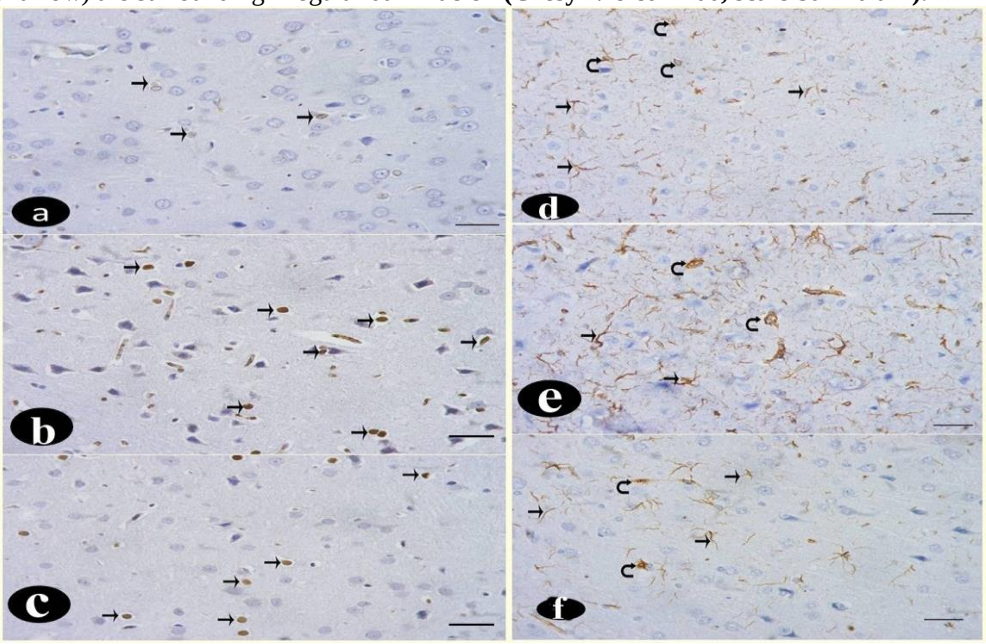

Figure (5): Photomicrographs of sections of immunoreaction with Caspase 3 in the cerebral cortex: **a-** The control group showing negative reaction to Caspase–3 antibody in the neurons. Few cells appear with weak nuclear reaction (arrow). **b-** The group exposed to hyperlipidemia showing most of neurons with strong nuclear reaction to Caspase–3 antibody (arrow). **C-** The hyperlipidemic+chyrsin group showing some neurons with moderate nuclear reaction to caspase–3 antibody (arrow). **(Caspase 3 immunostaining × 400, scale bar 20 um).** Photomicrographs of sections of immunoreaction with GFAP in the cerebral cortex: **d-**The control group showing weak cytoplasmic reaction in the glial cells with star-shaped appearance; slender bodies (curved arrow) and thin long processes (arrow).**e-** The group exposed to hyperlipidemia showing strong cytoplasmic reaction in the glial cells with hypertrophied cell bodies (curved arrow), and increased number and thickness of dendritic processes (arrow). **f-** The hyperlipidemic+chyrsin group showing moderate cytoplasmic reaction in the glial cells with less cell body hypertrophy (curved arrow), and thin processes (arrow**). (GFAP immunostaining × 400, scale bar 20 um).**

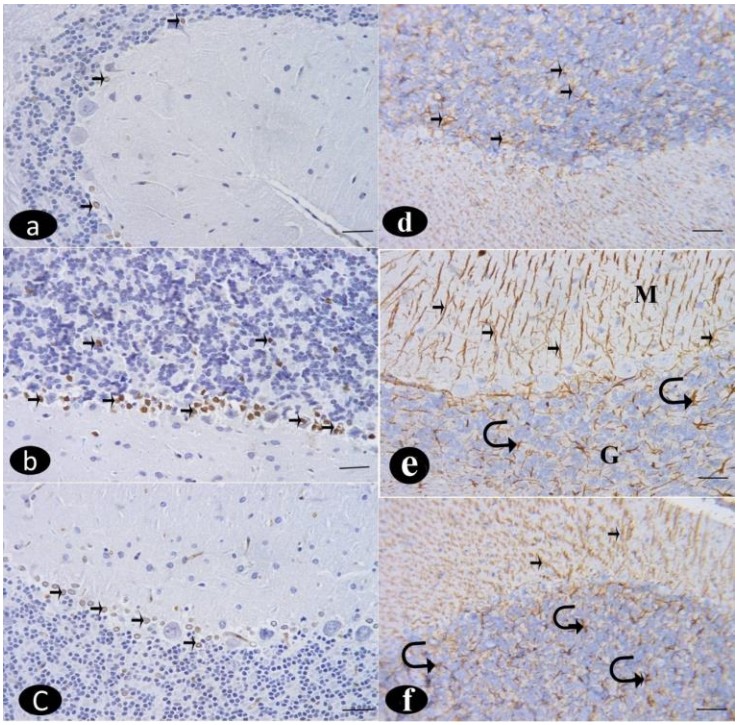

Figure (6): Photomicrographs of sections of immunoreaction with Caspase 3 in the cerebellum from: **a-** The control group showing negative reaction to caspase–3 antibody. Few cells appear with faint nuclear reaction (arrow). **b-** The group exposed to hyperlipidemia showing many neurons with strong nuclear reaction to caspase–3 antibody (arrow), mostly in the Purkinje and granular layers. **C-** The hyperlipidemic+chyrsin group showing some neurons with weak nuclear reaction to caspase–3 antibody (arrow). **(Caspase 3 immunostaining × 400, scale bar 20 um).** Photomicrographs of sections with GFAP immunoreaction in the cerebellum: **d-**The control group showing moderate cytoplasmic reaction in the glial cells which have thin processes (arrow). **e-** The group exposed to hyperlipidemia showing strong cytoplasmic reaction in the molecular layer (M), Bergmann-glia (modified astrocytes) (arrow) are perpendicular to the surface and parallel to each other but in the granular layer (G), true astrocytes appear star-shaped (curved arrow). **f-** The hyperlipidemic+chyrsin group showing moderate cytoplasmic reaction in the glial cells Note, Bergmann- glia (arrow) and true astrocytes (curved arrow). **(GFAP immunostaining × 400, scale bar 20 um).**

DISCUSSION

These days, the prevalence of metabolic syndrome (MS) is a sign of many underlying health problems, t he majority of which are caused by eating a highfat, highenergy diet (HFD). The metabolic syndrome m anifests as hypertension, dyslpidemia, hyperglycemi a, oxidative stress, and body fat accumulation. The brain, behaviour, and memory are all adversely affe cted, and the risk of dementia is raised [29]. Thus, w e investigated the effects of hyperlipidemia on the a dult male albino rat cerebellum and brain using bioc hemical, histological, and immunohistochemical me thods, as well as the possible protective effects of ch yrsin.

Aquino et al. [30] have proven that HFD affects blood brain barrier (BBB) permeability, causes brain oxidative stress, and triggers brain inflammation. They noted that inflammatory cytokines, ROS generation, and peripheral and central inflammatory indicators have all been increased.

In the present study, HFD rats showed a significant increase in serum cholesterol, TG and LDL compared to control group, while there was a significant decrease in HDL compared to control group. These results are similar to that stated by MA et al. [31]. Cerebral cholesterol and TG were positively correlated to serum cholesterol and TG that represent increase tissue TG with elevated TG and cholesterol in blood.

Also, we revealed increase cerebral oxidative stress in HFD rats rather than rats in control group presented by the significant decrease in antioxidant enzymes; reduced glutathione and superoxide dismutase, and by significant increase in cerebral MDA levels represented by the damage in the cell membrane induced by free radicals. These results are in line with other researchers who reported that hyperlipidemia causes increase in tissue MDA levels, and imbalance between free radicals and antioxidants resulting in brain tissue damage [32].

Several cytokines such as TNF-α, IL-1 and IL-6 are elevated in tissues when there is a pathological damage. The present study shows a significant increase in TNF-α, IL-1 and IL-6 in HFD rats compared to normal diet fed rats which approved that hypercholesterolemia promotes tissue damage represented by elevated cytokines. These results are in agreement with [33].

VEGF is elevated when there is damage in cerebral blood vessels, and its elevation indicates tissue inflammation and alteration in BBB permeability. Rats fed on HFD showed marked increase in cerebral VEGF level that indicates cellular damage, which are in line with [34]. On the other hand, hyperlipidemic+chrysin-treated group showed a significant decline in VEGF suggesting its anti-inflammatory and cytoprotective effects.

Also high cholesterol level leads to increase amyloid-β in cerebral tissue, an indicator to neuronal toxicity [35] which is in line with our results that show increase in amyloid-β in brain tissue of rats fed on HFD compared to normal diet group, while hyperlipidemic+chrysin-treated group reduced amyloid-β formation in brain tissue.

In the present study, chrysin was found to restore lipid profile and inflammatory cytokines in chrysin-treated rats compared to HFD group. These results could be attributed to its anti-inflammatory effects. Our results are in line with Tahereh et al. [36] who indicated that chrysin down-regulated inflammatory cytokines, and attenuated metabolic disorder indices. Also, chrysin restored the antioxidant parameters in treated group compared to HFD group.

In the present study, histopathological examination of the cerebral cortex of the group exposed to hyperlipidemia revealed neurons containing vesicular nuclei, and others with apoptotic nuclei characterized by neuronal shrinkage and chromatin condensation. Congested blood vessels were seen.

Also, the cortex of the cerebellum in the same group displayed Purkinje cells which had darkly pigmented pyknotic nuclei, and appeared shrunken. These results were consistent with Alkan et al. [37]. They noticed neuronal degeneration in the hippocampus in rats exposed to high-fat diet.

The vascular changes in the hyperlipidemic group particularly the congested blood vessels, were also similar to the results of Clarkson-Townsend et al. [38]. They demonstrated that HFD caused vascular alterations in the rodent retina that resulted in destroying of the retinal vascular endothelial cells besides, bleeding in those blood vessels. Some researchers hypothesised that this dilatation was caused by ischemia and hypoxia following HFD [39].

Examination of cresyl violet-stained sections in the cerebral and cerebellar cortices from the hyperlipidemic group revealed weak stained Nissel's granules in the cytoplasm of neurons and Purkinje cells. This finding was in line with the study done by Namavar et al. [40] who studied the effect of HFD on neuronal cells, and the volume of the mouse hypothalamus. Alkan et al. [37] concluded that HFD increases oxidative stress and causes neuronal degeneration in the hippocampus.

When the cysteine proteases of the caspase family, including caspase-3, are activated, a form of controlled cell death known as apoptosis occurs in tissues. Upon activation, the apoptosis indicator caspase-3 cleaves a number of proteins important for maintaining the structural integrity of the cell [41]. Cerebral and cerebellar sections immunostained with caspase-3 in HFD group of the current study showed intense reaction. The current finding is consistent with that of Mohamed [42] who investigated the impact of HFD on spleen. Hou et al.[43] confirmed HFD-induced apoptosis in the hippocampal neurons. They added that intake of HFD also makes the hippocampus more vulnerable to exaggerated proinflammatory reactions. Short-term HFD consumption has been shown to cause apoptosis, memory deficits, and the production of proinflammatory cytokines like interleukin-1 (IL-1) and tumour necrosis factor (TNF) in both humans and animals after only 3 to 10 days of exposure. Other data suggested that the mitogen-activated protein kinase (MAPK) pathway participates in the cellular responses to metabolic stresses such as HFD. MAPK signalling transduction contributes to the development of inflammatory biomarkers, gliosis, cell survival, and death in the cerebral and cerebellar cortices [44].

GFAP-immunostained cerebral and cerebellar sections of the hyperlipidemic group showed glial cells with hypertrophied with thicker dendritic processes. These results were confirmed morphometrically and statistically. Consistent with our results, some researchers have shown that HFD can cause the brain microglia and astrocytes, and neuroinflammation to increase. They discovered that GFAP considerably increased on days 14 and 21 following HFD. They hypothesized that

increased microglia and astrocytes may be responsible for the generation of IL-1. HFD activates microglia and astrocytes, which in turn cause neuroinflammation in the cerebral cortex [45].

Usage of chrysin in the current study ameliorated both the biochemical and the morphological changes induced by HFD administration. These findings matched those of Yuvaraj and his colleagues, who demonstrated that supplementing with chyrsin exhibited protective benefits against problems caused by the HFD in male rats myocardium [46].

Chyrsin is able to protect against neuronal cell damage by inhibiting oxidative stress and inflammation. According to other studies, chrysin anti-inflammatory and antioxidant capabilities reduce the severity of neuronal cell damage in mice with cerebral ischemic-reperfusion injury. Chrysin also has antioxidant effects on liver disease in rats. Chrysin antioxidant and anti-inflammatory qualities have been demonstrated in numerous studies to be effective at preventing neuronal cell damage and cognitive decline [47]. These properties would be a powerful effect of chrysin to ameliorate the histological changes in rats exposed to hyperlipidemia.

In conclusion, the present study demonstrated the anti-inflammatory and antioxidant protective effects of chrysin, which reversed the oxidative stress in rats exposed to hyperlipidemia.

Conflict of interest: The authors declare that there is no conflict of interest.

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Ethical approval:

All rats received care according to the standards of the National Guide for Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). The Institutional Animal Care and Use Committee (IACUC), Zagazig University, Egypt approved the design of the experiment.

Supplementary materials: Table S1-5

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Supplementary Materials:

Table S1: Mean values (± SD) of body weight and food consumption in the studied groups

	Control group	Hyperlipidemic group	Hyperlipidemic+ chrysin group
Body weight after 4 weeks	193±8.37	302±5.7*	218±18.23
Food intake g/day	23.16±0.27	23.29±0.28*	23.11±0.26

Values represent means ± standard deviation
P vaue<0.05 statistically significant.
Values with superscript star means significant difference

Table S2: Effect of chrysin on Serum level of cholesterol, TG, LDL and HDL presented as mean±SD

parameters	Control group	Hyperlipidemic group	Hyperlipidemic+ chrysin treated group
Cholesterol (mg/dl)	103.02±3.63	195.1±15.72*	104.8±4.5
TG (mg/dl)	84.4±4.9	134.2±9.16*	85.77±4.11
LDL(mg/dl)	38.7±1.98	57.6±2.87*	41.8±4.1
HDL (mg/dl)	49.1±2.36	36.2±2.53*	47.8±1.36

Values represent means ± standard deviation
P value <0.05 statistically significant.
Values with superscript star means significant difference.

Table S3: Effect of chrysin on cerebral level of cholesterol, TG, TNFα, IL6, IL1, amyloid beta (Aβ), VEGF, MDA, GSH and SOD presented as mean±SD

Parameters	Control group	Hyperlipidemic group	Hyperlipidemic+Chrysin treated group
Cholesterol (mg/g protein)	18.78±0.99	35.03±3.3*	20.9±3.2
TG (mg/g protein	1.8±0.21	4.2±0.41*	2.02±0.22
TNFα (ng/l)	190.75±75	230.57±5.74*	194.7±4.68
IL6 (ng/l)	146.6±1.32	195.4±4.21*	146±1.58
IL1 (µg/l)	162.3±2.12	249.4±4.47*	172.1±8.4
Aβ (µg/L)	1636.3±3.08	2205.9±33.8*	1678±22.2
VEGF (pg/L)	1145.66±4.8	2071.6±5.3*	1154.2±3.8
MDA (nmol/g tissue)	8.2±0.34	25.45±0.41*	7.9±0.36
GSH (mg/g tissue)	7.7±0.48	3.4±0.4*	6.9±1.1
SOD (U/g tissue)	14.5±0.42	3.5±0.47*	14.06±0.3

Values represent means ± standard deviation
P vaue<0.05 statistically significant.
Values with superscript star means significant difference

Morphometric and statistical results:

Table S4: Mean values (± SD) of morphometric parameters of the cerebral cortex in the studied groups

	Control group	Hyperlipidemic group	Hyperlipidemic+ chrysin group
Mean area % of GFAP	1.016±0.1106	3.28±0.1924*	1.198±0.2145
Optical density of Caspase3	0.364±0.0513	0.69±0.114*	0.402±0.0526

P value<0.05 statistically significant

Table S5: Mean values (± SD) of morphometric parameters of the cerebellum in the studied groups

	Control group	Hyperlipidemic group	Hyperlipidemic+ chrysin group
Mean area % of GFAP	5.94± 0.6656	14.7± 0.6245*	6.84±0.3507
Optical density of Caspase3	2.54±0.4159	8.88±0.3701*	3.34±0.2408

P value <0.05 statistically significant