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RESEARCH ARTICLE

THE POSSIBLE AMELIORATIVE EFFECT OF NIGELLA SATIVA SEEDS AGAINST HYPERLIPIDEMIA-INDUCED INJURY IN THE CEREBELLAR CORTEX OF RATS

Rasha A. Alshali[1,*] and Laila M. Aboul- Mahasen[1&2]

[1]Anatomy Department, Faculty of Medicine, King Abdulaziz University, Saudi Arabia
[2]Anatomy Department, Faculty of Medicine, Al-Azhar University, Egypt

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Corresponding Author:
Rasha A. Alshali

ABSTRACT

Hyperlipidemia describes abnormally high levels of lipoproteins, cholesterol, triglycerides in the blood. It is contributed by high fat diet (HFD) rich in saturated fatty acids, sedentary lifestyle plus other factors. HFD induces biochemical alteration associated with structural changes in the frontal cerebral cortex and the cerebellum of rats. This study evaluated the possible protective effect of Nigella sativa (NS) seeds powder on the biochemical and histopathological changes induced by HFD in rat's cerebellar cortex. It lasts 2 months and involved 24 rats separated into group I, control, group II, fed with HFD (20gm/100gm of diet/day), group III, fed with HFD plus treated with NS seeds powder (300mg/kg/day). After treatment, blood samples were collected for lipid profile assessment. Cerebelli were extracted and processed for histological examination. Body weight was increased in the hyperlipidemic group compared with the control group but reduced again by NS treatment. The serum lipid profile in hyperlipidemic rats showed significant improvement in rats fed on HFD with NS seeds powder. Microscopically, the cerebellar cortex of hyperlipidemic group showed marked degenerative changes in Purkinje cell layer with many swollen Bergmann cells. GFAP expression was abundant in the astrocytes of the three cortical layers. The cerebellar cortex of hyperlipidemia with NS-treated group showed marked improvement as most of the Purkinje cells had almost normal appearance and GFAP expression showed moderate expression as the control group. This study revealed significant biochemical results that are compatible to the histological and immunohistochemical findings. Thus, NS seeds had neuroprotective effects from hyperlipidemia-induced injury in the cerebellum.

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INTRODUCTION

Nowadays, the fast moving busy lifestyle distracts people from caring about their healthy habits and consumable diets like eating fresh fruits and green vegetables to boost their antioxidant status, adding to that consuming high fat diets and the insufficient physical activity and obesity. These factors collectively contribute to hyperlipidemia, which is a known risk factor leading to atherosclerosis and is commonly associated with many heart diseases, kidneys and pancreas illnesses (1, 2). Hyperlipidaemia is a medical disorder describing elevated levels of all or some lipoproteins, cholesterol, triglycerides in the blood, which can be correlated to elevated lipids. Hyperlipidaemia is contributed by high fat diet, inactive lifestyle, etc. High fat diets (HFD) are rich in saturated fatty acids that contributes to the development of hyperlipidemia (3).

WHO (2019) reported the top 10 causes of death, it has been stated that ischemic heart disease and stroke are the world's biggest killers, such diseases are still the leading causes of death globally in the last 15 years (4). High cholesterol induces neuro-inflammation in the brain which increases the risk of age-related neurological diseases(5-7).Rats receiving repeated treatment with cholesterol showed marked increase in the microglial immune-reactivity and elevated quantities of some inflammatory markers in the cerebral cortex, it also decreased the cholinergic neurons and caused impairment in cognitive function(8).Moreover, hypercholesterolemia disrupt the blood brain barrier in the cerebral cortex of rats evidenced by an increase of the anti-rat IgG immunoreactivity (9). Hypercholesterolemia is a chief risk factor for Parkinson's disease, Alzheimer's disease and vascular dementia(10, 11). Also, induction of high fat diet had injurious biochemical alteration accompanied with changes in the structure of the frontal cortex and the cerebellum of rats (12).

Indeed, it becomes essential to search for a protective approach to minimize the effect of hyperlipidemia and the related brain and neuronal complications. Since anciently, the medicinal natural herbs have been used widely and considered legally for the prevention or treatment of many human illnesses as they can change the path physiological processes in certain diseases. Recently, using the natural plants increased obviously compared with the chemical medications, because of many reasons, for instance it can be found and taken easily without prescription, its lower cost, no need for referral to healthcare professionals as well as trusting that treatments with natural products has fewer side effects. The WHO has predicted that around 80% of public got benefit from plant therapies (13, 14). Nigella sativa seed (N. sativa) is a plant cultivated in several areas in Asia and the Mediterranean countries, known as "Sannouj , Habbatel Baraka or Black seed". It has been used as a spice to a variety of Persian foods mainly in bread, salads, pickle and sauces. The chemical constituents of Nigella sativa seeds includes protein, carbohydrate, oil, fibers and saponin. The fixed oil chemical components of N. sativa are oleic acid, linoleic acid, palmitic acid, stearic acid, eicosadienoic acid, arachidic acid and myristic acid (15).

The major composites of N. sativa seeds are thymoquinone, pcymene, carvacrol, carvone, and thymol(16). The active constituent of N. sativa is principally thymoquinone (13.7%), which has potential variable therapeutic properties; it possess analgesic and anti-inflammatory activity seen in inflammatory conditions such as encephalomyelitis, colitis, edema and arthritis acting by suppressing inflammatory mediators like prostaglandins and leukotrienes (17, 18). N. sativa seeds have also antihistaminic effects and are used traditionally to treat cough asthma, bronchitis, headache, influenza, rheumatism and fever (19). Additionally, many studies demonstrated that N. sativa had anti-hyperlipidemic, anti-diabetic, anti-hypertensive effects (20-22), plus a potent antioxidative properties(23, 24). Previous studies revealed that N. sativa seeds may maintain the spatial cognitive challenged chronic cerebral hypoperfusion in rats(25, 26). Importantly, N. sativa seeds exhibited effective treatment of some neurodegenerative diseases. The thymoquinone constituent of N. sativa seeds shows anticonvulsant activity through intracerebroventricular injection (27) plus antiepileptic effect in the pilocarpine model of epilepsy (28). This study aimed to reveal the biochemical and histological alterations that occur in the cerebellar cortex in rats fed high fat diet and the potential protective effect of N. sativa seeds powder.

MATERIAL AND METHODS

Ethical approval: The study conduct and animal handling were authorized by the Ethics Committee of Biomedical Research-Faculty of medicine at KAU. The experiment was performed in agreement with the rules of dealing with experimental animals applied in King Fahd Medical Research Center (KFMRC).

MATERIAL

Nigella sativa seeds were purchased from a traditional market in Jeddah, Saudi Arabia.

Experimental animals: The albino rats were obtained from the Animal Experimental Unit of KFMRC, King Abdulaziz University (KAU), Jeddah, KSA. All rats were kept in well-ventilated cages and maintained in a controlled temperature (24°C ± 1°C), 55% ± 10% humidity with a 12/12 h light/dark cycle. They had free access to normal standard diet and water ad libitum.

Induction of hyperlipidemia: The albino rats fed with high fat diet (HFD) in a dose of 20 gm butter/100 gm diet (20%) (29) for eight weeks. Three weeks later, blood samples were collected for lipid profile assessment to confirm the induction of hyperlipidemia.

Experimental design: In this study, 24adult albino rats weighing (180 - 230 g) were used and the experiment lasts for eight weeks. The rats were allocated into three groups (n=8):

Group 1, control group, fed with standard diet.

Group II, hyperlipidemic (untreated) group, fed with HFD in a dose of 20 gm/100 gm per day (30).

Group III, treated group, fed with HFD (for 4 weeks) followed by treatment with Nigella sativa seeds powder at dose of 300mg/kg/day (for 4 weeks). The seeds powder were freshly prepared then suspended in water and the rats were fed300mg/kg body weight daily by intragastric tube (30). The body weight of the rats was recorded at the beginning of the experiment and two months later. After finishing the experiment, the rats were fasted for 10hours, with free access to water only.

METHODS

Blood analysis: After starting the experiment by 8 weeks, blood samples were collected and the sera were stored at-80˚C until analyzed for the biochemical analyses included triglycerides, cholesterol, high density lipoprotein HDL and low-density lipoprotein LDL.

Concentration of variable parameters in sera were measured by enzymatic immuno-assay methods using the commercially available kits: Rat Low-Density Lipoprotein (LDL)Elisa Kit(My Bio Source. com. San Diego. USA. Catalog No. MBS702165). Rat High-Density Lipoprotein (HDL) Elisa Kit (My BioSource.com .San Diego. USA. Catalog No. MBS2505957). Rat Triglyceride (TG) Elisa kit (My BioSource.com .San Diego. USA. Catalog No. MBS005097). Rat Quick Detect TM Total Cholesterol Elisa kit (My BioSource.com .San Diego. USA. Catalog No. MBS846775).

Technique for histological study: The rats were anaesthetized by diethyl ether inhalation. The heads of rats were opened, and the cerebelli were extracted and divided by sagittal cuts and fixed in 10% buffered formalin for histological and immunohistochemical examination.

Histological evaluation: For examination by light microscope, specimens were fixed in 10% formalin for 20 hours then processed to prepare cut paraffin-sections at a thickness of about 4-5 µm for hematoxylin and eosin stains(31)for general histological examination.

Immunohistochemical staining: The immunohistochemical staining for the GFAP protein localization was processed using the technique of avidin biotin peroxidase for detecting the anti-

glial fibrillary acidic protein (GFAP) to inspect the astrocytes. The paraffin sections were deparaffinized in xylene then rehydrated in a descending concentration of ethanol. They were immersed in PH 6 citrate buffer for 10 minutes for antigen retrieval followed by incubation for 18-20 hours with anti-GFAP monoclonal antibody (1:100 monoclonal mouse anti-GFAP)bought from (DAKOA gilent Technologies Company, Sigma ,USA). This special stain is for the intermediated filaments fibrillary acidic protein detected in astrocytes but not in nerve cells or other forms of glial cells such as microglia or oligodendroglia. The antibody was detected using abiotin-streptavidin detection system with 0.05% diaminobenzidine as a chromogen (Amersham, Little Chalfont, UK) and counterstaining was processed with hematoxylin (32). GFAP positive cells were identified by brown color of the cell membrane and the cytoplasm of astrocytes, and showed blue nuclei (33, 34). Finally, the slides were scanned by Digital pathology slide scanner (Philips IntelliSite Pathology Solution). Photographs were captured from the scanned slides (Alborgdigitalpathology.com, KSA).

Statistical analysis: The results were analyzed using SPSS statistical software, version 19.0 (SPSS Inc., Chicago, IL, USA). The differences between the results were analyzed using one-way ANOVA. Data was reported as means ± standard deviations (SD). Differences were considered statistically significant if $P<0.05$.

RESULTS

BODY WEIGHT GAIN: The body weight of the rats was recoded at starting the experiment and two months later, data is presented in table 1. As showed, body weight was increased as a result of HFD supplementation in comparison with the control group. But it was reduced in the third group after treatment with NS seeds powder.

Table 1. Comparison of body weights (grams) in different studied groups

	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
G1 (control group)	194.75± 3.59	205.75 ±5.75	228.50 ±6.40	245.50 ±9.03	258.50 ±7.85	276.25 ±14.52	280.00 ±9.63	289.25± 20.27
G2 (Butter group)	240.00± 8.68	261.50 ±15.50	277.00 ±19.24	287.00 ±22.32	315.25 ±19.00	327.00 ±19.61	345.25 ±21.09	355.75± 24.74
Significance	[1]P= 0.0001	[1]P= 0.0001	[1]P= 0.0001	[1]P= 0.002	[1]P= 0.001	[1]P= 0.002	[1]P= 0.001	[1]P= 0.002
G4 (Butter & Nigella sativa group)	216.25± 6.50	220.75 ±7.50	237.25 ±22.53	249.25 ±19.55	258.75 ±22.77	277.50 ±26.15	283.25 ±31.79	298.50± 33.04
Significance	[1]P= 0.0001	[1]P= 0.040	[1]P= 0.380	[1]P= 0.755	[1]P= 0.987	[1]P= 0.932	[1]P= 0.852	[1]P= 0.646

Data are expressed as mean+/- standard deviation. [1]P: significance versus G1 (Control group). *n=8*

BIOCHEMICAL RESULS: The assessed lipid profile concentrations are presented in table (2).The levels of serum triglyceride, cholesterol and LDL were significantly higher in Group 2 fed on HFD than those of Group 1 ($P< 0.001$). However, rats in Group 3 (fed on HFD and treated with Nigella sativa seeds) showed a significant decrease in the serum levels of triglyceride, cholesterol and LDL as compared with G2 ($P < 0.001$).

HISTOLOGICAL RESULTS: The H&E stained sections from the cerebellar cortex of control rats G1 (Fig. 1) showed

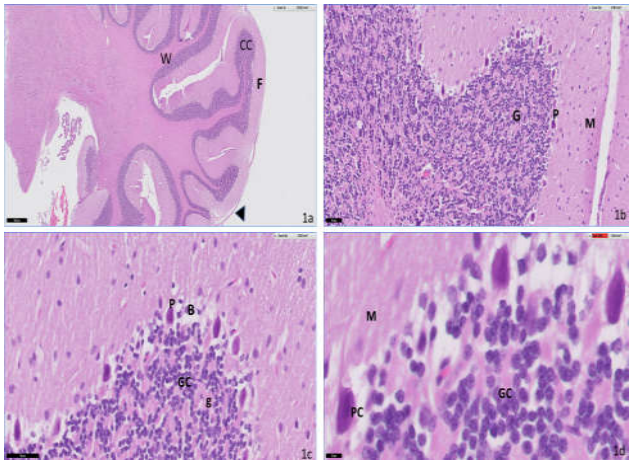

Figure (1): Photomicrographs of cerebellar cortexstained with H and E from control group showing:
1a. Normal architecture of the folia (F) surrounded by a layer of pia matter (▲). Each folium is formed of a central core of white matter (W) and an outer cerebellar cortex (CC). 1b. Normally arranged three cortical layers of cerebellar cortex, molecular layer (M) with few neurons, one row of pear-shaped purkinje cell layer (P), and inner granular layer with dense closely packed nuclei (G). 1c. The nuclei of Bergmann cells (B), the astrocytes of the purkinje layer (P) are shown around the purkinje cell bodies. Notice the cerebellar glomeruli (g) occupying the spaces between the granule cells (GC). 1d. Large pyriform shaped Purkinje cells (PC) having basophilic granular cytoplasm and vesicular nuclei with apical dendrites branching into the molecular layer (M). Show also numerous small granular cells with rounded darkly stained nuclei (GC). *H&E stain 1a X 20 1b X200 1cX400 1d X1000*

the normal structure of folia protected by the pia matter. Each folium is formed of a white matter as a central core and a gray matter as an outer cortex; the cerebellar cortex. The cerebellar cortex showed three layers from the outermost to the innermost: a molecular layer, a Purkinje cell layer and agranular layer. The outer molecular layer composed of nerve fibers and few small, scattered cells. The middle Purkinje cell layer contained Purkinje cells arranged in one row between the molecular and granular layers. They were pyriform in shape with dendrites on the apex that were branching into the covering molecular layer. Their nuclei were large rounded and vesicular with prominent nucleoli. The Purkinje cells were enclosed by few Bergmann's astrocytes. The inner granular layer contained abundant crowded small granular cells with darkly stained rounded nuclei. The cerebellar glomeruli formed of complex glial and neuronal processes appeared as scattered acidophilic regions in between the granule cells. Visualization of H & E stained sections from the cerebellar cortex of rats in G2 fed on HFD (Fig.2) showed that in spite of the pattern of folia was preserved, degenerative changes mainly in Purkinje cell layer was found. Distortion of Purkinje cells and cellular shrinkage or complete destruction was detected in addition to many swollen Bergmann's cells were seen around the purkinje cells. Some Purkinje cells showed deep staining of the cytoplasm and pyknotic, ill-defined nuclei and other Purkinje cells had homogenized cytoplasm and faint nuclei. The molecular and granular layers showed minimal affection**.** Some of the granular cells had pale nuclei in between the clumping deeply stained nuclei. The blood vessels of the cerebellar cortex appeared dilated and congested. Visualization of H & E stained sections from the cerebellar cortex of rats G3 fed on HFD and N. sativa seeds powder (Fig.3) showed that the architecture of the cerebellar cortex was greatly improved

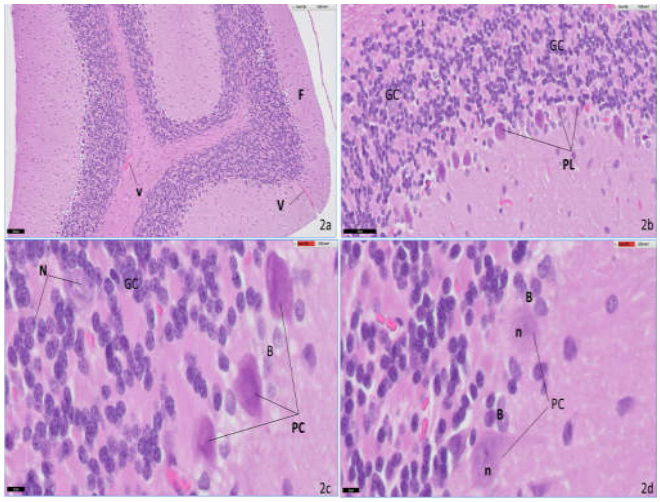

Figure (2): Photomicrographs of cerebellar cortexstained with H and E from rats fed on HFD showing: 2a. Preservation of the folia pattern (F). Notice dilated and congested blood vessels (V). 2b. Distortion and shrinkage of the Purkinji cell layer (PL). 2c. Some Purkinje had deeply stained cytoplasm and pyknotic, ill-defined nuclei (PC), swollen Bergmann's cells and some cells of granular cells (GC) had pale nuclei (N) in between the numerous deeply stained nuclei. 2d. Other Purkinje cells (PC) had homogenized cytoplasm with faint nuclei (n) and swollen Bergmann's cells (B). ***H&E stain 2a X 100 2bX400 2cX1000 2dX1000***

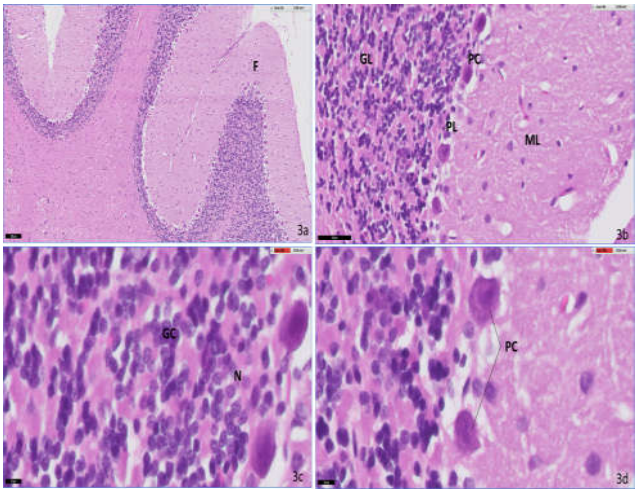

Figure (3): Photomicrographs of cerebellar cortex stained with H and E from rats fed on HFD and Nigella sativa seeds powder showing: 3a. Normal architecture of the cerebellar cortex into folia (F). 3b. Mild disorganization of the Purkinje cell monolayer (PL) as most of the Purkinje cells had almost normal appearance (PC). The molecular layer (ML) and granular layers (GL) appeared more or less as the control group. 3c. Numerous small granular cells with rounded darkly stained nuclei (GC). Some cells of the granular layer had pale nuclei (N). 3d. The Purkinje cells (PC) had pyriform shape with pale vesicular nuclei (n) and prominent Nucleoli as the control cells. ***H&E stain 3a X 100 3bX400 3cX1000 3dX1000***

and appeared very similar to the control group as a mild disorganization of the Purkinje cell monolayer. Most of the Purkinje cells appeared almost normally. They were pyriform in shape having pale colored vesicular nuclei with prominent nucleoli. Few shrunken with deep stained Purkinje cells were observed in between the normal cells. The molecular and granular layers appeared similar to the control group but some cells of the granular cell layer had pale nuclei plus few congested blood vessels were seen.

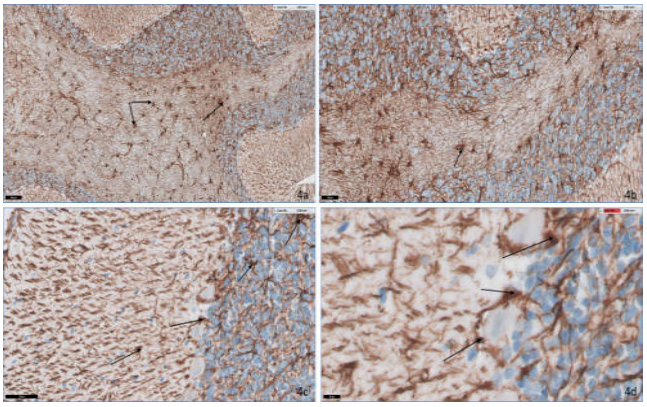

Figure (4): Photomicrographs of GFAP-immunostained cerebellum sections of the control rat group showed:

4a. Moderate positive expression in the astrocytes in the white matter of the cerebellum 4b. Moderate positive expression in the astrocytes in the white matter core of the folia. 4c. Moderate positive cytoplasmic expression in the cell bodies and processes of the astrocytes (arrows) in the three cortical layers. 4d. Moderate positive cytoplasmic expression in the cell bodies and processes of the astrocytes (arrows) in the Purkinje cell layer (Bergmann's cells). ***GFAP 4aX100 4bX200 4cX400 4dX1000***

IMMUNOHISTOCHEMICAL RESULTS: GFAP-immunostained cerebellar sections of the control group showed moderate positive cytoplasmic expression in the cell processes and the cell bodies and of the astrocytes in the three cortical layers, in the white matter core of both the folia and the cerebellum (Fig.4). In group fed on HFD, the number of GFAP positive astrocytes showed marked increase and were seen as large abundant GFAP immune-expression in the three cerebellar cortical layers,in the white matter core of the folia and in the white matter of the cerebellum (Fig. 5).

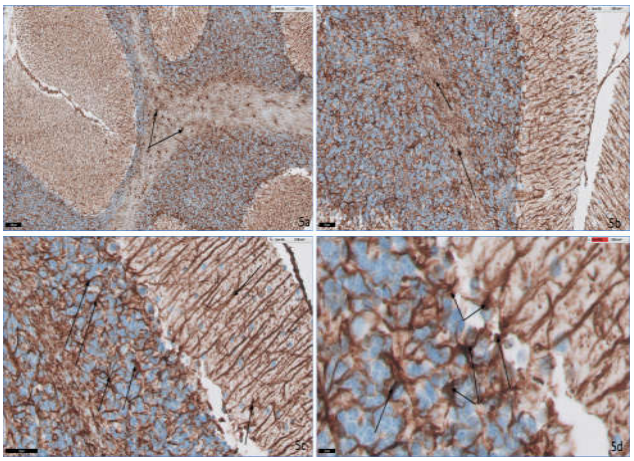

Figure (5): Photomicrographs of GFAP-immunostained cerebellum sections of the rat group fed on HFD showing a marked increase in the number of GFAP positive astrocytes

5a. Abundant large GFAP positive expression in the astrocytes in the white matter of the cerebellum 5b. Abundant large GFAP positive expression in the astrocytes in the white matter core of the folia 5c. Abundant large GFAP positive cytoplasmic expression in the cell bodies and processes of the astrocytes (arrows) in the three cortical layers. 5d. Abundant large GFAP positive cytoplasmic expression in the cell bodies and processes of the astrocytes (arrows) in the Purkinje cell layer (Bergmann's cells) ***GFAP 5aX100 5bX200 5cX400 5dX1000***

In group fed on HFD and Nigella sativa seeds powder showed a moderate GFAP immune-expression in the astrocytes appeared more or less as the control group (Fig. 6).

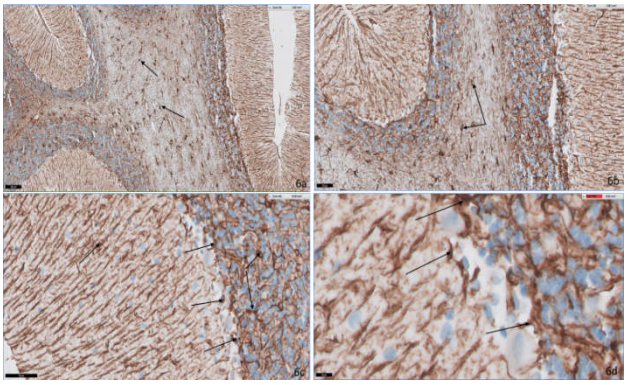

Figure (6): Photomicrographs of GFAP-immunostained cerebellum sections of the rat group fed on HFD and Nigella sativa seeds powder showed: 6a. Moderate positive expression in the astrocytes in the white matter of the cerebellum 6b. Moderate positive expression in the astrocytes in the white matter core of the folia 6c. Moderate positive cytoplasmic expression in the cell bodies and processes of the astrocytes (arrows) in the three cortical layers. 6d. Moderate positive cytoplasmic expression in the cell bodies and processes of the astrocytes (arrows) in the Purkinje cell layer (Bergmann's cells). *GFAP 6aX100 6bX200 6cX400 6dX1000*

	G1 (control)	G2 (HFD)	G3 (HFD + N. sativa seeds)
Triglyceride(mg/dl)	72.5 ± 5.3 ***	107 .69 ± 1.4 ***	60.69 ± 1.4 ***
Cholesterol (mg/dl)	84.4 ± 3.1***	212 .53 ± 2.7 ***	100.53 ± 2.62***
LDL-C (mg/dl)	47.6 ± 0.6 ***	151 .87 ± 2***	64 .87 ± 2.4***
HDL-C (mg/dl)	29.4 ± 0.5 ***	40 .01 ± 0.2 ***	25 .01 ± 0.19***

Data are presented as mean+/- standard deviation. (*** $p < 0.001$)Significance versus G1 (Control group); ($^{###}p < 0.001$)Significance versus G2 (HFD), $n=8$.

Table (2). Effects of N. sativa seeds supplementation on blood lipid profile

DISCUSSION

In this study, an increase of the body weight was noticed in the hyperlipidemic group compared with control group, but that was lowered after the treatment with NS seeds powder. Similar findings were reported in many similar studies (35-37). This study showed that the high fat diet significantly increased serum levels of triglycerides, total cholesterol and LDL in comparison to the control group, which is also compatible with previous studies (3, 30, 37).High cholesterol level was considered a major risk factor of many neurological diseases (7, 9, 11). In this work, improved serum lipid profile was observed in hyperlipidemic rats fed on Nigella sativa seeds powder, as the total cholesterol, LDL-cholesterol and triglycerides were significantly reduced. A study revealed that Nigella sativa showed significant improvement in the lipid profiles in women after menopause (declined total cholesterol, LDL-cholesterol and TG, and increased HDL-cholesterol) more than the treatment with placebo within two-month intervention (22). Other study reported that Nigella sativa seeds had a beneficial effect on lipid profile in type II diabetic patients. The study demonstrated that patients ingested 2 gm/day of Nigella sativa showed a significant decrease in total cholesterol and TG compared with their baseline records and control patients(21).The mechanism of Nigella sativa in decreasing lipid profile (TC, TG, LDL-cholesterol and VLDL-cholesterol) possibly due to its components of monounsaturated fatty acids and phenols. N. sativa seeds were rich with linoleic acid and thymoquinone that was providing the protective antioxidant effect (30, 38). In the present study, the cerebellar cortex of hyperlipidemic group showed marked degenerative changes mainly in Purkinje cell layer. This layer was disturbed and shrink age leaving empty spaces around them. Most of the Purkinje cells show deep staining cytoplasm with pyknotic, ill-defined nuclei. Some Purkinje cells with homogenized cytoplasm and faint nuclei were also noticed**.** Many swollen Bergmann cells were seen. The molecular and granular layers showed minimal affection in the form of appearance of numerous vacuolated areas near Purkinje layer**.** Most of the granular cells had pale nuclei although some granular cells had clumping deeply stained nuclei. The capillaries of the cerebellar cortex appeared dilated and congested.

These results were confirmed in many studies. Shrinkage of the cerebellar cells and significantly reduced survival cells count beside elevated LDH, lipid peroxide and nitrite levels were detected in the cells that confirmed neurodegenerative changes with cholesterol and LPS challenges (12, 33, 39). There are variable suggested mechanisms of neurotoxicity induced by hypercholesterolemia. It has been reported that hypercholesterolemia may cause injury to the endothelial cells lining the arteries and capillaries leading toa reduction in the blood flow, an impaired metabolism, and a lower nutrition and levels of oxygen in the brain, consequently increasing the probability of cognitive disorders. A paper sated that hypercholesterolemia badly affects the function of endothelial cells in brain microvessels and possibly predisposing to cerebral infarctions (11). These results confirms our findings which showed dilation of the blood vessels. The high fat diet is closely related to vascular injury and oxidative stress thus it was possible that the development of the neurodegenerative disease is induced by hypercholesterolemia through increased oxidant production. Many researchers established that oxidative stress had a chief role to initiate the development of atherosclerosis through the stimulation of inflammation and production of cytokines. Inflammation was a common pathway in several neurodegenerative diseases (40). The present study revealed a significant decrease in the number of Purkinje cells with their shrinkage leaving empty spaces around them. These findings are in agreement with a study mentioned that Purkinje cell density is decreased in neurodegenerative disorders as Alzheimer's disease (41).

In this study, GFAP cytoplasmic immune-expression in the astrocyte's cell body and processes revealed a significant increase in the number and size of astrocytes in hyperlipidemic rats compared to the control group. GFAP is the main protein of the intermediate filament of the mature astrocytes, thus, considered as an astrocyte specific marker. Previous researchers assumed that any degenerative brain insult induce astrocyte proliferation and hypertrophy with increased GFAP production resulting in severe astrogliosis. The appearance of these reactive astrocytes due to neuronal damage may be considered as a compensatory process following neurodegeneration (33). The activated astrocytes stow variable neurotrophic factors for neuronal survival(7), even though, obvious activation of astrocyte proved by increased expression

of GFAP had been related with the production of inflammatory cytokines, reactive oxygen species and a modification in the extracellular space (42). It is well known that excessive cholesterol could lead to rigidity and loss of membrane fluidity in neurons plus creation of cell debris, this can explain the Astrocytosis. The produced cell debris could act as antigen and initiate inflammatory reaction and/or gliosis(39). The amyloid plaques, a pathologic hallmark of Alzheimer's disease, were concomitant with GFAP positive activated astrocytes (43). In this work, examination of H & E-stained sections from the cerebellar cortex of hyperlipidemia with Nigella sativa seeds group showed an obvious improvement of the cerebellar cortex as most of the Purkinje cells almost appeared normally, showing pyriform shape with pale vesicular nuclei and prominent nucleoli. Few shrunken and deeply stained Purkinje cells were observed in between the normal cells. The molecular and granular layers appeared almost like the control group, but some cells of the granular cell layer had pale nuclei and few congested blood vessels were seen. These results suggested the Nigella sativa seeds possess a neuroprotective action.

These results were in agreement with many other studies. The Thymoquinone (TQ) derived from Nigella sativa extract reduced the neuronal degeneration (44). Researches had revealed that TQ had a potential analgesic action via stimulating the opioid receptors in the central nervous system (45, 46).One study showed that intracranial administration of TQ stopped PTZ-induced seizures and this action is possibly mediated through increasing the GAB Aergic system tone as well as opioid receptors(27).Abdul-Zaher *et al*. have described that Nigella sativa oil has a protective effect on mice from the tolerance and dependence induced by tramadol as it has a prospective therapeutic role via blocking the excessive production of drug-induced nitric oxide and oxidative stress (47).

CONCLUSION

This study showed histological and immunohistochemical findings that were in parallel with the biochemical results, thus, it could be concluded that Nigella sativa seeds powder had a potential protective effect against hyperlipidemia and neurodegeneration, and they may protect the cerebellum against hyperlipidemia-induced damage. Therefore, it is highly recommended to increase the daily consumption of Nigella sativa seeds in our diets.

Conflicts of interest: The authors have declared that no conflicts of interest exist.

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