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ORIGINAL ARTICLE

THE EFFECT OF CYCLOPHOSPHAMIDE ON HIPPOCAMPAL STRUCTURE OF ADULT MALE RATS (ROLE OF ROSUVASTATIN)

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Summary

Background: Limited researchs were noticed on the histological impact of cyclophosphamide on rats' brains and the reports on the effects of antioxidants to protect these harmful effects are scanty. Trials have assessed the effect of statins in cancer regarding the association between statins use and cancer incidence.
Aim: To investigate the protective and ameliorative effects of rosuvastatin on the brain toxicity induced by a single dose of cyclophosphamide in male rats.
Materials and methods: Twenty-four rats were divided into 3 groups (n=8 for each). The control group includes animals which were received no treatment for 15 days. The cyclophosphamide group includes rats which were received a single dose of 150 mg/kg cyclophosphamide intraperitoneally on day 8 of the experiment, then left for 7 days without treatment. The rosuvastatin+ cyclophosphamide group enrolled rats which were gavaged with rosuvastatin (20 mg/kg/day) for 7 days and then they have received an injection of cyclophosphamide and gavaged with the same dose of rosuvastatin for other 7 days. All rats were subjected to euthanasia. Brain from each case was extracted and prepared for histological examination.
Results: The hippocampal sections of rats which were belonged to group 2 showed some alterations including the presence of cells with ghost appearance and damaged neurons. Features of dense nuclei of damaged hilar cells were manifested with evidence of extracellular vacuoles besides some pyknotic nuclei in these sections. Hippocampal sections of rats of group 3 showed that the majority of pyramidal cells and granule cells manifested seminormal appearance with improvement in the thickness of both granule and pyramidal cell layers.
Conclusions: Rosuvastatin has a protective and ameliorative role against the adverse effect of cyclophosphamide on rat hippocampus which may be useful in clinical practice of cancer treatment.

Key words: cyclophosphamide; rosuvastatin; hippocampus; dentate gyrus; rat

Introduction

Many cancers are treated by different approaches including chemotherapy. In general, there are nonspecific effects of chemotherapy as these agents affect the normal tissues besides its action on cancer cells directly

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or indirectly (1). Moreover, nurses and pharmacists are exposed in their occupation to many drugs throughout their production, and distribution including anticancer (2). The various spectrum of neurological effects was shown in cases of cancer as adverse effects of chemotherapy (as cyclophosphamide (CP)), including memory defects, general confusion, short time of concentration, and cognitive problems (3). The effect is mainly on the cerebral cortex and hippocampus even a single dose (4) and these changes may be irreversible.

One of the rare areas that manifested neurogenesis (which is vital for memory and learning) is the hippocampus (5). The neuronal progenitors (in the sub-granular zone of the dentate gyrus (DG)) divide to produce new neurons during life, which is responsible for integration as neural circuits (6).

Cyclophosphamide can induce more harmful action on the healthy cells as its uptake was higher in these cells (7). There is a different mechanism by CP on cells including oxidative stress by phosphoramide mustard and acrolein metabolite of it (8). Free radicals can be produced by CP with inhibition of many enzymes that have antioxidant properties besides the alkylating effect on deoxyribonucleic acid -DNA, especially in the dividing cells, which leads to initiation of apoptosis in different tissue including the brain as CP can pass through the blood-brain barrier (BBB) (9).

In addition, a suggestion of the effects of chemotherapy on the axis between the pituitary gland and gonads via gonadotropin-releasing hormone (GnRH). This hormone (which is important for neuronal activity) was detected in cornu ammonis (CA) of the hippocampus and the cerebral cortex (10).

Several studies discussed the beneficial effects of exogenous antioxidant agents to reduce the impact of many toxic substances (11) and atorvastatin and rosuvastatin (synthetic statins) are among them (12, 13). The statins lead to a reduction in plasma lipoproteins' level and cholesterol (14, 15), as they inhibit enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (16). Furthermore, statins have a crucial role as antioxidants and anti-inflammatory agents without dependency on the reduction of cholesterol levels. These effects are shown even at low doses of them (17). Statins have a protective role in renal damage, gonadal toxicities (18), cardiac toxicity, and gastrointestinal tract toxicity (19) by their prevention of the reduction in endogenous antioxidant enzymes. Additionally, it modulates the platelet and lymphocyte count (20, 21), modulate glycemic profile (22).

At present, approximately, clinical trials (n=160) are assessing the impact of statins in cancer, both as a therapy and as a biomarker regarding the association between statin use and cancer incidence (23). Limited works were noticed on the histological impact of cyclophosphamide on rats' brains and the reports on the effects of antioxidants to protect these harmful effects are scanty. This study aims to investigate whether rosuvastatin has protective and ameliorative effects on the brain toxicity induced by a single dose of cyclophosphamide in male rats via histological techniques.

Materials and Methods

According to the rules of the Ethical Committee of Medical Researches /College of the Medicine/University of Mosul (UOM/COM/MREC/21-22 (36) on 22/02/2022), a total of 24 Wister-albino rats were used. They were purchased from the animal house /College of Veterinary Medicine / University of Mosul. They were housed in convenient animal conditions, ten rats per cage. Food and water were freely allowed. In a ventilated region with suitable temperature (22–25°C), all rats were subjected to a cycle of 12 hours of light to dark. After acclimatization of seven days (24), the animals were divided into 3 groups as follows (25, 26):

1. The first group (control) includes eight animals which were received no treatment for 15 days.
2. The second group(cyclophosphamide) includes eight rats which were received a single intraperitoneal dose of 150 mg/kg CP on day 8 of the experiment then left for seven days without treatment. Vials of cyclophosphamide monohydrate (Endoxan, Baxter Oncology GmbH (Kantstrasse, Germany)) was used. 500 milligram of CP as a dry powder in each vial. Distilled water was used to dissolve CP(27).
3. The third group (rosuvastatin+ cyclophosphamide) includes eight rats which were gavaged with rosuvastatin (20 mg/kg-AstraZeneca, United Kingdom) for seven days, and then they have received a single intraperitoneal injection of CP, then they were gavaged with rosuvastatin (20 mg/kg/day) for other seven days.

Study termination

All rats were subjected to euthanasia by using ether after 24 hours of the final dose of administration (using a piece of cotton wool was soaked with ether and was put in a desiccator just before the introducing of the rat into the desiccator for 5 minutes) (24).

Then both cerebral hemispheres were separated after the brain bring out. After that, cutting of the posterior portion of the brain that was contained the hippocampus was done to be put in 10 percent of neutral buffered formalin for one week. Dehydration of specimens followed by clearing and embedding in paraffin was conducted to be sectioned with a microtome at five micrometers in thickness to be prepared for histological examination via light microscope using hematoxylin and eosin (10, 27).

Results

Hippocampal sections of rats of group 1 were examined via light microscope and they revealed the presence of two regions including the hippocampus proper and the DG.

Four zones were shown in the hippocampus proper (cornu ammonis 1, cornu ammonis 2, cornu ammonis 3, and cornu ammonis 4) with the dorsal location of cornu ammonis 1 and cornu ammonis 2, while the descending arch was made by cornu ammonis 3 that continues till the beginning of the hilar region of the DG (between two blades of DG). Cornu ammonis 4 is located inside the hilar region of the DG. (Figure 1 A). Histologically, three layers were seen in all zones of hippocampus proper sections: polymorphic, pyramidal, and molecular (Figure 1B). The pyramidal cells from the middle layer of the hippocampus proper and each cell contain a large vesicular nucleus with the obvious nucleolus. Few neurocytes were arranged in the internal molecular and external polymorphic layers (Figure 1B).

In addition, among all sheets of the hippocampus proper, identification of glial cells(interneurons) was recorded. These interneurons have dense nuclei and deep cytoplasm. (Figure 1B).

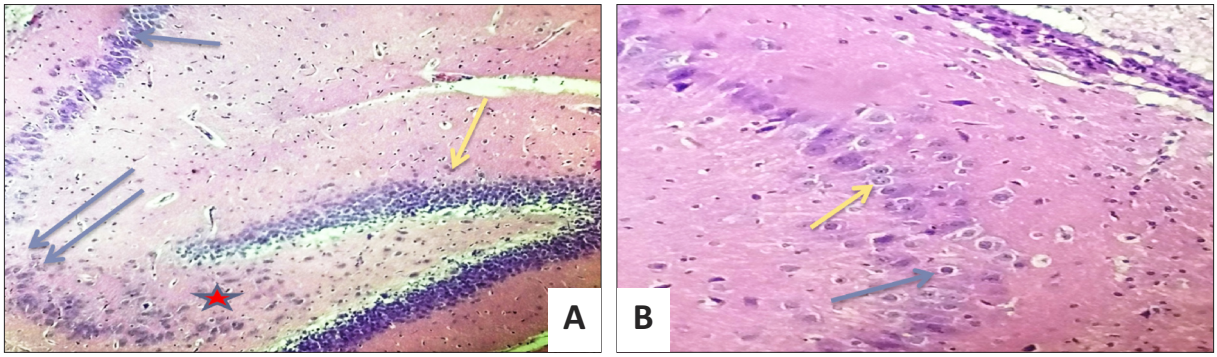

Figure 1. A microphotograph of a rat's hippocampus of the group. (A)The cornu ammonis (single arrow), cornu ammonis (double arrows), and cornu ammonis (star) are seen. V-shaped dentate gyrus is noticed (yellow arrow) (250X). (B) The normal appearance of cornu ammonis with three layers polymorphic, pyramidal, and molecular is noticed. The pyramidal cell looks normal (yellow arrow) with an interneuron (single arrow) (400X). hematoxylin and eosin stains (H and E).

In DG, the pyramidal cell layer was substituted by the granule cell layer. (Figure 2A). Regarding the DG, features of damaged neurons with dense nuclei in the granular cell layer were noticed with evidence of gliosis is noticed (Figure 2B). The current work revealed that hippocampal sections of rats which were belonged to group 2 showed some alterations including disorganization of the pyramidal cell layer with a decrease in the pyramidal thickness of that layer and presence of ghost-like cells (Such cells are characterized by light cytoplasm and nuclear swelling and karyolysis) and damaged neuron (Figure 2C). Accumulation of cytoplasmic vacuoles in the degenerated cells that lost the cellular marks. Furthermore, nuclear pyknosis (small and deep) was shown in several pyramidal necrotic cells with mini perineural spaces and scattered healthy neurons.

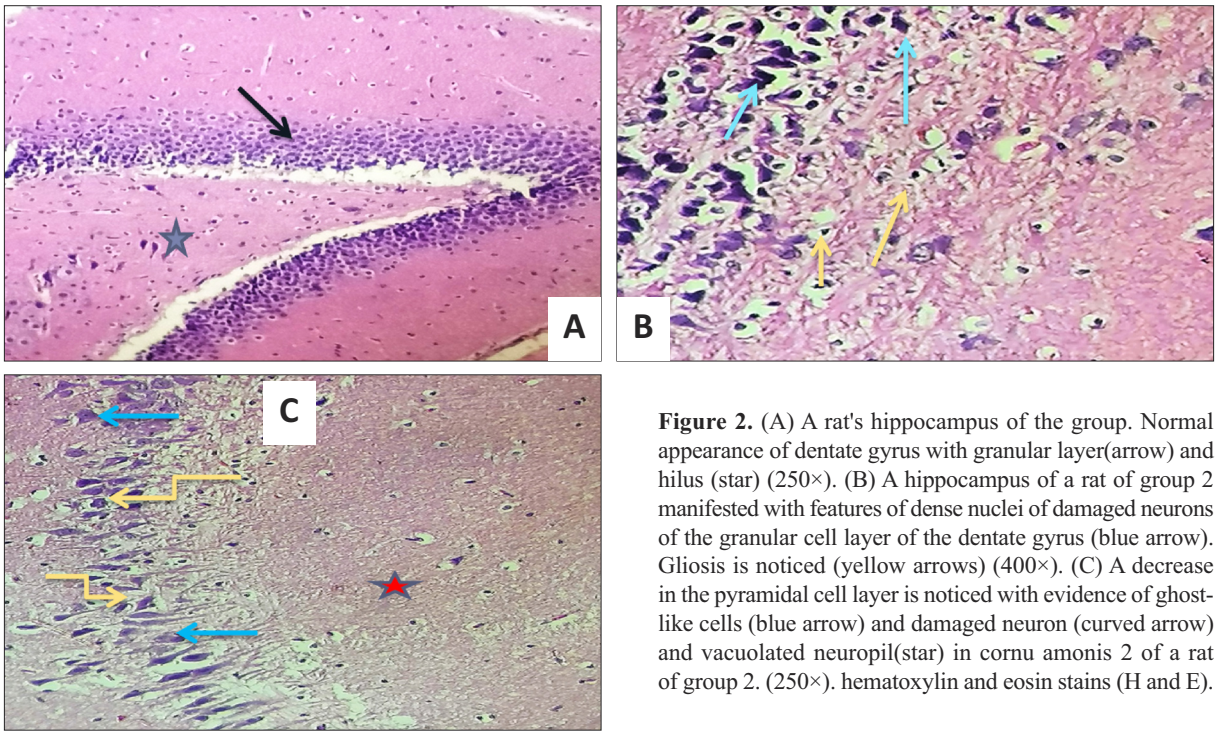

Figure 2. (A) A rat's hippocampus of the group. Normal appearance of dentate gyrus with granular layer(arrow) and hilus (star) (250×). (B) A hippocampus of a rat of group 2 manifested with features of dense nuclei of damaged neurons of the granular cell layer of the dentate gyrus (blue arrow). Gliosis is noticed (yellow arrows) (400×). (C) A decrease in the pyramidal cell layer is noticed with evidence of ghost-like cells (blue arrow) and damaged neuron (curved arrow) and vacuolated neuropil(star) in cornu amonis 2 of a rat of group 2. (250×). hematoxylin and eosin stains (H and E).

There is a decrease in the granular cell layer of DG sections from the rat of group 2 (Figure 3 A, B). The hippocampal sections of rats belonging to group 2 revealed the presence of vacuolation in the granular cell layer of the DG (Figure 3C).

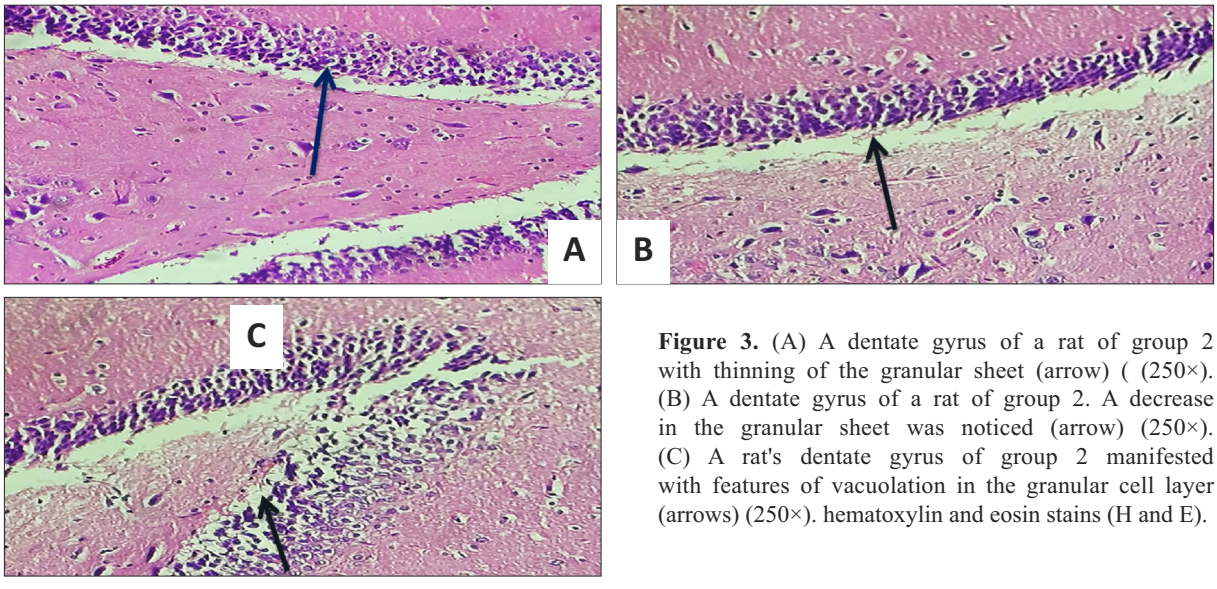

Figure 3. (A) A dentate gyrus of a rat of group 2 with thinning of the granular sheet (arrow) ((250×). (B) A dentate gyrus of a rat of group 2. A decrease in the granular sheet was noticed (arrow) (250×). (C) A rat's dentate gyrus of group 2 manifested with features of vacuolation in the granular cell layer (arrows) (250×). hematoxylin and eosin stains (H and E).

Concerning the hilus, features of dense nuclei of damaged hilar cells of DG were manifested with evidence of extracellular vacuoles, with some pyknotic nuclei and gliosis (Figure 4A). Some ghost-like hilar cells were identified (Figure 4B).

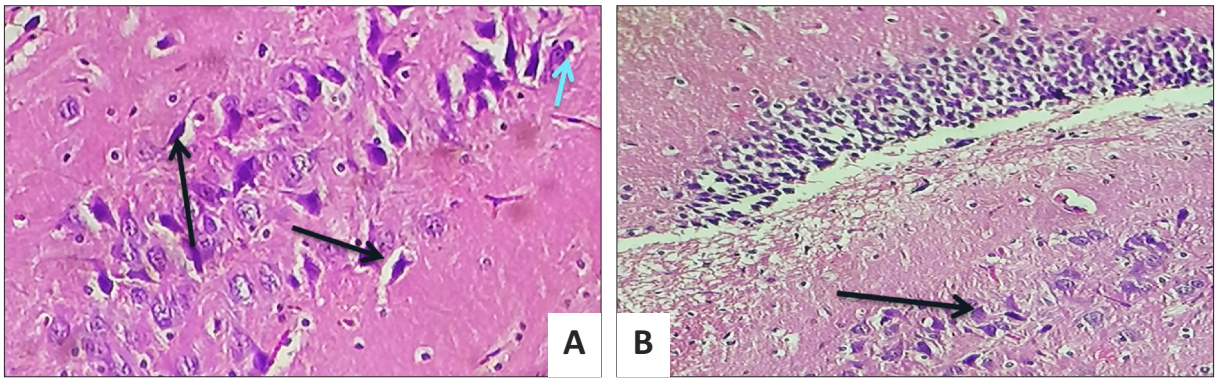

Figure 4. (A) A hippocampal section from the rat of group 2 manifested with features of dense nuclei of damaged hilar cells of dentate gyrus with extracellular vacuoles (black arrow) or pyknotic (blue arrow) and gliosis (400×). (B) A hippocampal section from the rat of group 2 manifested with features of ghost-like hilar cells of the dentate gyrus (arrows) (400×). Hematoxylin and eosin stains (H and E).

Examination of hippocampal sections of rats of group 3 showed that the majority of pyramidal cells and granule cells manifested seminormal appearance as large cells with vesicular nuclei, however, some cells showed features of pyknosis among these healthy cells (Figure 5) with better improvement in both pyramidal cell layer thickness and the granule cell layer thickness.

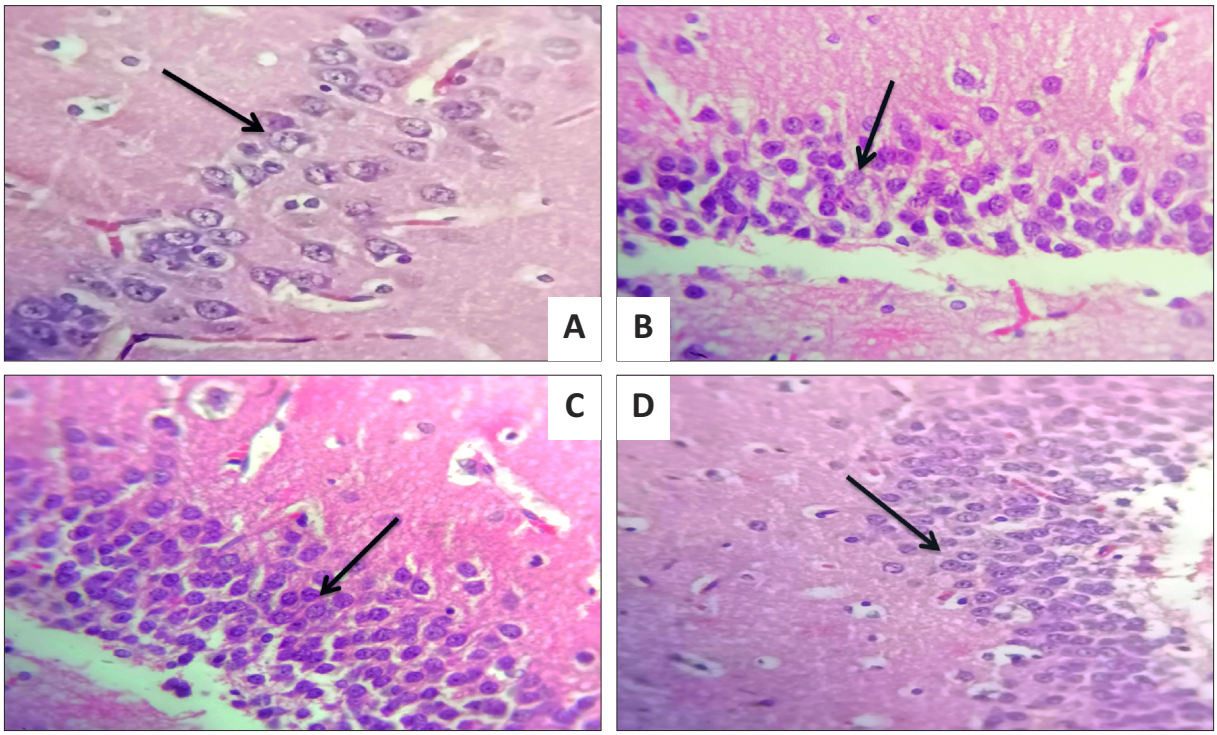

Figure 5. (A) A hippocampal section from the rat of group 3 showed the semi-normal appearance of neurons (arrows) of the large vesicular nucleus with obvious nucleoli. Nissl granules showed normal peripheral distribution (400×). (B) Features of the near-normal appearance of neurons in a granular sheet of the dentate gyrus (arrows) in a hippocampal section from the rat of group 3 (400×). (C) A Dentate Gyrus from a rat of group 3 manifested with features of the near-normal appearance of neurons in granular sheet (arrows) (D). A Dentate Gyrus with semi-normal neuronal appearance in granular sheet (arrows)in a hippocampal section from the rat of group 3 manifested (400×). Hematoxylin and eosin stains (H and E).

Discussion

Cyclophosphamide has been regarded as the cornerstone drug for cancer treatment. Substantial efforts were conducted to produce a chemotherapeutic agent with more efficiency and lower toxicity, however, little attempts were done considering ameliorating their deleterious side effects (28). Due to the toxic effects of chemotherapeutic drugs on healthy cells, there is a critical restriction in the clinical outcome of such agents. For this reason, the development of adjuvant treatment was required to amplify the efficacy versus lessening the related side effects (29, 30).

Many factors are contributed to neurogenesis ablation and lead to cognitive impairment including irradiation (31) cytotoxic drugs including - CP, and hippocampal lesions (32). There are alkyl crosslinks (that are caused by CP and its metabolites) that occur inside and among DNA strands of dividing cells and lead to apoptosis (33).

The dose of cyclophosphamide that is used in this study was based on a study conducted by Branda et al., which is lower than the anticipated median lethal dose of 200mg/kg (30). The current investigation revealed that hippocampal sections of rats which were belonged to group 2 showed some alterations including disorganization of the pyramidal cell sheet with a decrease in the thickness of the pyramidal cell layer and presence of ghost-like cells and damaged neurons with pyknotic nuclei indicated its neurotoxic effects. These observations were similar to those of other studies (33, 10) as CP was associated with marked hippocampal alterations involving the neuroses (apoptotic, necrotic, and dystrophic) and consequently the thinning in neurocytes cells of pyramidal and granule sheets (10) beside the defect in the neurogenesis which is responsible for the disability in memory and cognition (32).

Furthermore, there is a decrease in the granular cell layer of the dentate gyrus in a hippocampal section from the rat of group 2 with features of damaged neurons in the granular cell layer and hilus of the dentate gyrus, and evidence of gliosis is noticed. Pyknosis was seen in these sections with extracellular vacuoles in the granular cell layer. These observations were in accordance with those of others (10). An immunohistochemical study (34) confirmed that CP induced marked changes via apoptosis and dystrophic changes in the neurocytes of the hippocampus beside the cerebral cortex.

Over the last 30 years, the number of survivors from different types of malignancies has raised as there are highly effective anti-cancer protocols. However, the majority of these cases were left with at least one side effect of the chemotherapeutic agents that are considered as serious (10). CP has a neurotoxic effect, basically, due to its ability to generate the reactive oxygen species inside the cells (27, 35). The clarification of the mechanisms that stand behind the cognitive impairment and neurotoxic effects of chemotherapeutic drugs may help in preventing these adverse effects of such agents in patients with cancer (36).

The present study revealed that there is protecting and ameliorating role of rosuvastatin as shown via light microscopic examination of hippocampal sections of rats which were gavaged with rosuvastatin for seven days before and after the exposure to CP. A previous reports suggested that statin has a neuroprotective role due to its antioxidant action (37).

Several studies suggested the role of antioxidants in ameliorating the deleterious effect of CP on different organs including the hippocampus (13, 32, 38-41). Authors suggested the role of edaravone (antioxidant) in reversing the alterations in acetylcholine esterase activity alternation and in ameliorating the changes in behavior and brain structure after exposure to CP (40).

It has been suggested that atorvastatin has a hepatoprotective role against both ovarian and hepatic injury after exposure to CP as atorvastatin is an antioxidant and anti-apoptotic agent (8, 13).

Conclusion

Limited works were noticed on the histological impact of CP on rats' brains. The hippocampal sections of rats which were exposed to CP showed some alterations in their histology. Hippocampal sections of rats were

administered with rosuvastatin before and after CP exhibited seminormal appearance in their histology. In conclusion, this work revealed that rosuvastatin may protect and mitigate the rat hippocampus from the adverse effect of CP and this may be useful in the clinical practice of cancer treatment.

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Conflict of Interest

The authors declare that no conflict of interest exists for this research.

Adherence to Ethical Standards

The study was approved by the Medical Research Ethics Committee in the university of Mosul, the study approval number and date UOM/COM/MREC/21-22 (36) on 22/02/2022.

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