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Methotrexate Toxic Effects on the Cerebellum and Vitamin C Protective Function

Hala Mohamed AlKhalidi[1], Shatha Shayan Almutairi[2], Ali Hassan A. Ali[3,4*]

[1]*Clinical Pharmacy Department, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia*
[2]*College of Medicine and Medical Science, Arabian Gulf University, Manama, Kingdom of Bahrain*
[3]*Basic Medical Science Department, College of Medicine, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia*
[4]*Anatomy Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt*

Abstract

***Background*:** The cerebellum, often referred to as the "little brain," is primarily responsible for motor coordination and balance. However, recent research has shown that it also plays a role in a variety of cognitive processes, such as learning, attention, and even emotion regulation. This study aimed to evaluate how vitamin C might lessen the negative effects of methotrexate on the cerebellum of male albino rats.

Methods and Results: The study included 30 healthy adult albino rats weighing between 200 g and 250 g. The animals were divided into three groups. Group 1 included 10 rats that did not receive medicine and were given distilled water orally and regularly. Group 2 included 10 rats given intraperitoneal injections of 10 mg/kg methotrexate once a week for 4 weeks. Group 3 included 10 rats given intraperitoneal injections of 10 mg/kg of methotrexate once a week for 4 weeks and 20 mg/kg of vitamin C via gastric gavage every other day for four 4 weeks. On the designated day, the animals were killed, and the cerebellum was removed and prepared for light microscopic analysis.

In the methotrexate-treated group, the granular cell layer in the cerebellar layer was noticeably thinner than the molecular cell layer, which had significantly less cellularity. Purkinje cells lost their flask-shaped arrangement and mono-laminar configuration. Purkinje cells were irregular and shrunken, with several vacuolated patches between them and homogeneous, darkly pigmented cytoplasm and weak nuclei.

In the methotrexate + vitamin C group, the thickness of the cerebellar cortex layers was better preserved, and the molecular layer displayed normal cellularity. Certain Purkinje cells had typical dimensions, shape, and organization, whereas other cells displayed uneven and homogenous cytoplasm, with less discernible vacuolated regions surrounding them. The molecular cell layer had greater cellularity, was regularly shaped, had more plentiful basket cells, and had no perineural gaps around them.

Conclusion: Using vitamin C can lessen the toxicity of methotrexate, which is extremely toxic to the cerebellar cortex and destroys cortical cells, particularly granule cells and Purkinje cells.(**International Journal of Biomedicine. 2024;15(1):196-199.**)

Keywords: methotrexate • cerebellum • antioxidant • vitamin C

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Introduction

The cerebellum, often referred to as the "little brain," is primarily responsible for motor coordination and balance. However, recent research has shown that it also plays a role in a variety of cognitive processes, such as learning, attention, and even emotion regulation.

Methotrexate is a potent folic acid antagonist and cytotoxic agent that prevents cell division. In addition to being an anti-proliferative and cytotoxic drug used to treat inflammatory disorders and autoimmune problems like rheumatoid arthritis, it is employed in treating several forms of malignant tumors.[1] Methotrexate is a drug that crosses the blood-brain barrier and damages neurological tissue by

generating ROS, lowering antioxidant enzyme levels, and inducing oxidative stress, which results in apoptosis, tissue destruction, and neurotoxicity.[2] There has been growing evidence of methotrexate-related neurotoxicity during the past ten years.

Excessive dosages of methotrexate cause harm to the central nervous system, including decreased hippocampus neurogenesis, white matter demyelination, and cognitive impairment. Nervous tissue has a higher concentration of vitamin C than other organs. It plays a significant role in the nervous system's proper operation and helps the brain's antioxidant defense system. It is crucial for neurotransmission and neural development.[3] Due to the correlation between methotrexate-induced neurotoxicity, oxidative stress, proliferative inflammation, and an elevated neuro-immune response, it has been proposed that substances with strong anti-inflammatory, antioxidant, and proven neuroprotective properties, like vitamin C, may be effective in halting or reducing the onset of methotrexate-induced neurotoxicity.[4] Vitamin C therapy has been demonstrated to mitigate neuropathological alterations, memory impairments, and neurodegenerative changes in rats exposed to neurotoxic agents.[5]

This study aimed to evaluate how vitamin C might lessen the negative effects of methotrexate on the cerebellum of male albino rats.

Materials and Methods

The study included 30 healthy adult albino rats weighing between 200 g and 250 g. Methotrexate (25 mg/mL injection solution) (Mylan Pharmaceutical Company) was given as an injectable. After being diluted in three milliliters of saline, one milliliter contains 25 mg of methotrexate. C-Retard vitamin C (capsules of 500 mg) (Hikma Pharmaceuticals Company) was dissolved in 10 mL of pure water, and 50 mg of vitamin C in 1 mL of water was used for the experiment.

Animals were raised at the Faculty of Pharmacy under conventional feeding and temperature settings. The animals were divided into three groups. Group 1 included 10 rats that did not receive medicine and were given distilled water orally and regularly. Group 2 included 10 rats given intraperitoneal injections of 10 mg/kg methotrexate once a week for 4 weeks. Group 3 included 10 rats given intraperitoneal injections of 10 mg/kg of methotrexate once a week for 4 weeks and 20 mg/kg of vitamin C via gastric gavage every other day for 4 weeks.

After the experiment was over, the animals were anesthetized with ether inhalation, killed, and their skulls opened. The cerebellum was removed, cut into sagittal sections, and promptly fixed in 10% formalin to create serial paraffin slices. After staining with H&E, specimens were viewed under a light microscope. The cerebellar specimens were fixed in a phosphate buffer containing 2.5% glutaraldehyde for 3 hours. A phosphate buffer was used to wash fixed-tissue samples before they were post-fixed in 1% osmium tetroxide. Semithin sections one um thick were cut using an ultra-microtome, picked up on gelatinized glass slides, and dyed with toluidine blue following dehydration in increasing alcohol grades and embedding in EPON.

Results

Group 1: The cerebellum comprised many folia, with small sulci dividing one folium from the others. The cerebellar cortex, exterior gray matter, and the medulla, or inner white matter, combined to produce each folium. The cerebellar cortex comprises three layers: deep basket cells, many deep fibers, and a sparse number of superficial stellate cells. Large and grouped in a single mass, Purkinje cells had a flask-shaped cytoplasm with pale basophilic vesicular nuclei that were prominently nucleoli. The cerebellar glomerulus, a tiny acidophilic region, was located between the closely packed, highly pigmented nuclei of granular cells (Figure 1).

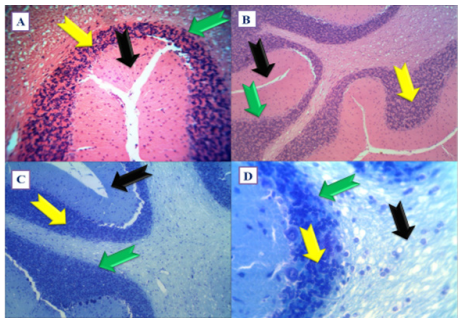

Fig.1. *Different images of the cerebellum of the control group. The thickness of the three layers that make up the cerebellar cortex: the granular layer (yellow arrows), the Purkinje cell layer (green arrows), and the molecular layer (black arrows). (A) and (B): H&E, ×100 magnification; (C) Toluidine blue, ×100 magnification; (D) Toluidine blue, ×400 magnification.*

Group 2: The granular cell layer in the cerebellar layer was noticeably thinner than the molecular cell layer, which had significantly less cellularity. Smaller-sized perineural gaps were visible around basket cells in the molecular layer. Purkinje cells lost their flask-shaped arrangement and mono-laminar configuration. Purkinje cells were irregular and shrunken, with several vacuolated patches between them and homogeneous, darkly pigmented cytoplasm and weak nuclei. Granule cells exhibited aberrant clumping and dispersion; more cerebellar glomeruli were found (Figure 2).

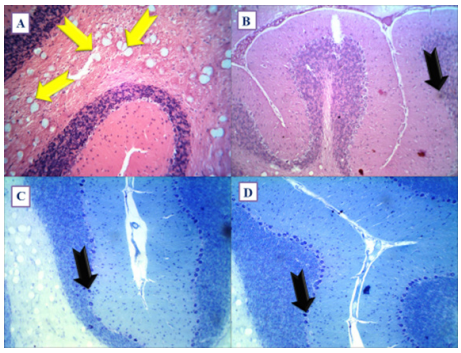

Fig.2. *Different images of the cerebellum of the methotrexate-treated group. Purkinje cells are amorphous, shrunken, and have vacuolated gaps between cells (yellow arrows) and homogenized, darkly stained cytoplasm degenerated cells (black arrow) and vague nuclei. (A) and (B): H&E, ×100 magnification; (C) and (D): Toluidine blue, ×100 magnification.*

Group 3: The thickness of the cerebellar cortex layers was better preserved, and the molecular layer displayed normal cellularity. Certain Purkinje cells had typical dimensions, shape, and organization, whereas other cells displayed uneven and homogenous cytoplasm, with less discernible vacuolated regions surrounding them. The molecular cell layer had greater cellularity, was regularly shaped, had more plentiful basket cells, and had no perineural gaps around them. The granular cell layer displayed properly distributed cells with less identifiable cerebellar glomeruli (Figure 3).

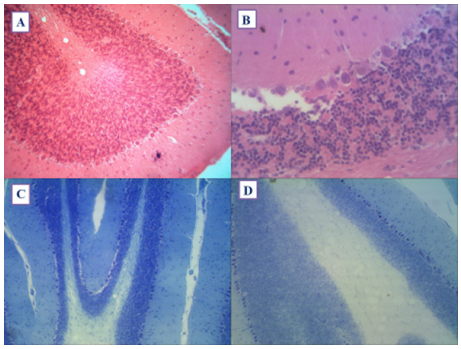

Fig.3. *Different images of the cerebellum of the methotrexate + vitamin C group. While most cells have typical size, shape, and organization, some have homogenous cytoplasm, ill-defined nuclei, less noticeable vacuolated regions, and more granule cells than others. (A) and (B): H&E, ×100 magnification; (C) and (D): Toluidine blue, ×100 magnification.*

Discussion

The cerebellum is an essential organ for emotion, thought, and motor function. Due to its hypothesized role in movement planning, adaptation to unique circumstances, and long-term memory storage, the cerebellar cortex has become the subject of extensive research.[6] One cytotoxic drug in an adjuvant chemotherapy cocktail is methotrexate. It is a folic acid antagonist used to treat psoriasis and rheumatoid arthritis, two non-neoplastic diseases.[7] Positive outcomes with methotrexate focus attention on its effects after treatment; the neurological system is significantly harmed by chemotherapy.[8] Numerous theories have been proposed on the effects of oxidative stress on the central nervous system. It arises from an imbalance between the production of ROS and the defenses of antioxidants.[9]

Water-soluble and naturally occurring as an antioxidant, vitamin C shields human organs from oxidative damage. It prevents cytotoxicity and is a good scavenger for radicals that damage membrane lipids.[10]

The cerebellar cortex layers of the research specimens treated with methotrexate exhibited a noticeable thinning, resulting in a deformation of the normal architecture. Purkinje cells had a pyriform shape, shrank unevenly, had weak nuclei and dark, homogeneous cytoplasm, and displayed vacuolated regions. Granule cells had many cerebellar glomeruli separating them from shrunken, deeply pigmented nuclei.

These findings were consistent with a previous study that found Purkinje cells in rats treated with methotrexate had reduced size, lost their characteristic form, and some had developed squamous cells. Another study that used methotrexate on pigs revealed that the Purkinje cells shrank, lost their flask shape, and had numerous gaps surrounding them.[11]

In the current study, light microscopic examination of the group treated with methotrexate and vitamin C revealed a slight restoration of normal architecture, thickness of layers in the cerebellar cortex, and appearance of Purkinje cells with a regular flask shape, arranged in a regular row with pale basophilic cytoplasm, distinct nuclei, and less visible spaces between them. Cerebellar glomeruli were not as well defined, and the granule cells were closely packed with transparent cytoplasm. These results were consistent with a previous study that discovered that, under a light microscope, rat cerebellar cortex specimens that had been given vitamin C with monosodium glutamate had maintained their histological structure but that a small percentage of Purkinje cells and granular cells had shrunk.[12]

Vitamin C is an electron donor that can return in a reduced state and combines with superoxide, hydroxyl radicals, and oxygen singlets to minimize cell damage.[13] Because the brain produces more ROS than other organs, consumes a lot of oxygen, has a high concentration of polyunsaturated fatty acids, and has low levels of antioxidant enzymes, it is especially susceptible to oxidative stress, which is avoided by antioxidants like vitamin C.

In conclusion, using vitamin C can lessen the toxicity of methotrexate, which is extremely toxic to the cerebellar cortex and destroys cortical cells, particularly granule cells and Purkinje cells.

Ethical Approval

The experiments followed the protocol approved by the Institutional Animal Care and Use Committee at the Prince Sattam bin Abdulaziz University Institutional Review Board (SCBR-093-2023).

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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***Corresponding author:** *Prof. Ali Hassan Abdou Ali. Basic Medical Science Department, College of Medicine, Prince Sattam bin Abdulaziz University, Al-Kharj 11942, KSA. E-mail: alihassan3750@yahoo.com*