

Toxic Effects of Methotrexate on Cerebellar Cortex: Review Article

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ABSTRACT

Background: Cerebellum, also known as the "small brain," is a highly stereotypical cortical structure located in the vertebrate hindbrain. Its primary function is to aid in motor control, but it also processes a wide range of sensory functions. Methotrexate (MTX) is widely used as a chemotherapy agent for treating many different kinds of cancer. Psoriasis, SLE, IBD, vasculitis, and a wide variety of other connective tissue illnesses can all benefit from it without any negative side effects. The neurological system is particularly vulnerable to methotrexate's adverse effects. Although neuronal symptom is also frequently observed. Since MTX-induced neurotoxicity has received less attention than its other side effects, its causes remain unknown. **Objective:** This review article aimed to assess the possible toxic effects of Di Ethyl phthalate (Methotrexate) on cerebellar cortex. **Methods:** Methotrexate, cerebellar cortex were all looked for in PubMed, Google scholar, and Science direct. References from relevant literature were also evaluated by the authors, but only the most recent or complete study from January 2000 to May 2022 was included. Due to the lack of sources for translation, documents in languages other than English have been ruled out. Papers that did not fall under the purview of major scientific investigations, such as unpublished manuscripts, oral presentations, conference abstracts, and dissertations, were omitted. **Conclusion:** Purkinje cell shrinkage, karyolytic alterations in granule cells, and nuclear damage were observed as pathological changes at the cellular component of the cerebellar cortex after methotrexate exposure.

Keywords: Methotrexate, Cerebellar Cortex.

INTRODUCTION

The cerebellum is found in the back of the skull, in the posterior fossa. In front of it are the pons, medulla, and fourth cerebral ventricle. The tentorium cerebelli and all cerebellar connections with other areas of the brain pass through the pons, which is separated from the overlying cerebrum by a layer of leathery dura mater⁽¹⁾.

The cerebellum is divided into the anterior lobe, the posterior lobe, and the flocculonodular lobe, each of which has a specific physiological function. Fissures called the major fissure and the posterolateral fissure separate these lobes. The spinocerebellum is made up of the middle section of the cerebrum's anterior and posterior lobes. The cerebrocerebellum, also known as the neocerebellum, is located in the brain's lateral zone⁽²⁾.

Histology of the cerebellum:

The molecular cell layer is the outermost, followed by the Purkinje cell layer and finally the granule cell layer, which is the innermost and most primitive layer of the cerebellar cortex. Flattened Purkinje cell dendritic trees and a vast array of parallel fibers from the granule cell layer, which enter the Purkinje cells' dendritic trees at right angles, make up the molecular cell layer⁽³⁾. Stellate cells and basket cells, two types of inhibitory interneurons, can also be found in the molecular cell layer. The form of stellate cells resembles a star. They are the only type of neuron present in the outer and middle thirds of the cerebellar cortex's molecular layer. Their dendritic and axonal architectures allow for the following categorizations: Cells in the upper third of the molecular layer have short, circinate, twisted dendrites and small axonal fields, whereas deep stellate cells have long axons with

thin, varicose collaterals and radiating, twisted dendrites⁽⁴⁾. The nuclei of superficial stellate cells are spherical, and their cytoplasm is almost completely occupied by one or more shallow indentation(s), whereas the cell bodies of deeper stellate cells might be significantly larger and have more copious cytoplasm. Compared to the outer stellate cells, these ones are more ellipsoidal in shape, and they often exhibit complex nuclear indentations. Except for a thin, uneven border condensation, chromatin is typically evenly disseminated throughout the karyoplasm. Additionally, the nucleolus is typically located near the periphery of the cell⁽⁵⁾.

When compared to larger neurons, stellate cells have very little cytoplasm. Unruly protrusions from the nuclear envelope frequently fuse with the rough endoplasmic reticulum. Rosettes of ribosomes can be seen dispersed throughout the membrane-free zones and indentations of the nucleus, as well as in the rough endoplasmic reticulum. Golgi apparatuses, however, show a great deal of variation in their arrangement from one cell to the next ones⁽⁶⁾.

Multiple "stellate" (sparsely branched) dendrites are characteristic of the molecular layer's stellate cells. Stellate cells have synaptic contacts with parallel fibers, climbing fibers and basket cell axons. The axon of a stellate cell is a distinctive process that grows in the center and outer thirds of the molecular layer, and it is directed toward the secondary and tertiary dendritic branches of the Purkinje cell. A complex neuropil formed by the Purkinje-parallel and climbing fiber spine synapses, surrounded by the Bergmann glial cell cytoplasm is observed adjacent to the stellate neurons⁽⁷⁾.

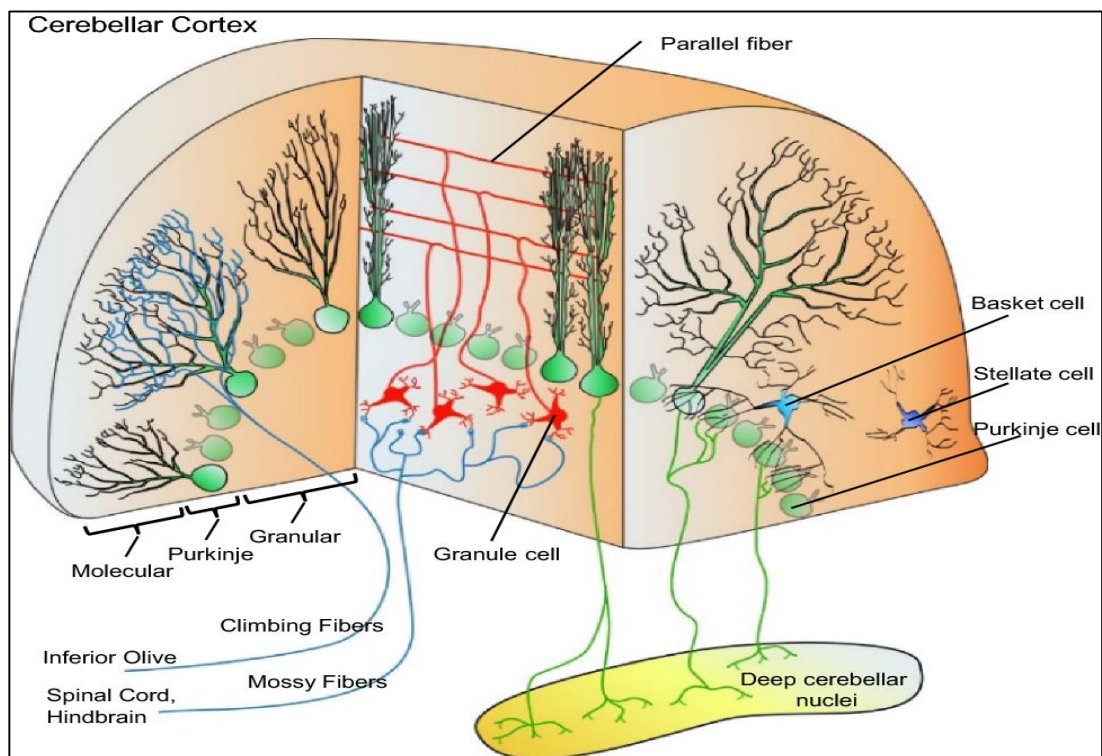


Figure (1): Layers and cells of cerebellar cortex ⁽⁸⁾.

Basket Cells (Basket neurons) are buried within the molecular structure. Soma of a basket cell is spherical, with a big, irregular, oval nucleus whose primary axis is perpendicular to the Purkinje cell layer. It's an open structure with chromatin that's spread out in all directions. The nuclear envelope can sometimes be found in close proximity to blocks of condensed chromatin. It's not uncommon for the nucleolus to be located on the periphery and to be contained within one of these blocks. Besides a few spots, the nuclear envelope is sloppy and devoid of ribosomes ⁽⁹⁾.

Golgi apparatus, lysosomes, multi vesicular bodies, vesicles, small mitochondria, cisternae of the rough endoplasmic reticulum, and small clusters of unbound ribosomes are all found in the sparse cytoplasm. Microtubules start to form in clusters near the nucleus. The dendrites typically converge at their bases in a parallel array before branching out into processes. The apical dendrites of basket cells are short and unbranched, and they project directly into the molecular layer. While the majority of dendrites are spine-free. Those that do contain spines create extensive contacts with neighboring Purkinje cells. Purkinje cells nest in a basket-like structure formed by the lateral and radial smooth axons of basket cells, which run parallel to the purkinje perikarya ⁽¹⁰⁾. Generally speaking, basket cells are larger and more spherical, while stellate cells are smaller and more elliptical. Stellate cells predominate in the upper half of the molecular layer, while basket cells are more common in the lower half ⁽¹¹⁾.

The cell nuclei of Bergmann glial cells and Purkinje cells are located in this thin layer, also known

as the Purkinje cell layer. Purkinje cells are one of the earliest discovered types of neurons and are widely considered to be the brain's most distinctive. In 1837, anatomist Jan Evangelista Purkinje was the first to describe them ⁽¹²⁾.

Purkinje cells are big pear-shaped cells that form a single layer. Their cell bodies are the most prominent and distinctive in the cerebellum at the microscopic level. They have larger nuclei than the granule cells that occupy the innermost layer. There is a preponderance of basophilia in their cell bodies and dendrites. Their distinctive dendritic tree structure sets them apart. The dendrites are extremely branched out, but they're badly flattened in a plane that's perpendicular to the cerebellar folds. Purkinje cells have their dendrites in the molecular layer, and their axons extend into the granule cell layer, where they form synapses with neurons in the deep nuclei of the cerebellum ⁽¹³⁾.

Methotrexate:

The chemotherapy drug methotrexate (MTX) is a popular option for treating many different kinds of cancer. Psoriasis, SLE, IBD, vasculitis, and many other connective tissue diseases can also benefit from it safely and effectively ⁽¹⁴⁾.

Methotrexate is an antifolate antimetabolite that inhibits the growth of cancer. Methotrexate-polyglutamate is formed when it is taken up into the cell by carriers known as the human reduced folate carriers. Together, methotrexate and methotrexate-polyglutamate block the enzyme dihydrofolate reductase, which is responsible for converting inactive

dihydrofolate to the metabolically active tetrahydrofolate ⁽¹⁵⁾.

Tetrahydrofolate is required during the production of both DNA and RNA nucleotides. De novo purine synthesis is blocked by methotrexate-polyglutamate because it inhibits purine and thymidylate synthase. The cytotoxic effect of this pathway is exploited in the therapy of cancer. Nausea, vomiting, mucosal ulcers, and loss of appetite are the most often reported negative side effects, most people experience them, and it is simple to treat them ⁽¹⁶⁾.

Methotrexate's folic acid supplementation can prevent these side effects because they are comparable to those of folate insufficiency. Neurotoxicity is a serious side effect of MTX. Though age, coexisting diseases, and genetic susceptibility can amplify the occurrence of side effects. Dose, treatment duration, and dose intensity are the primary determinants of neurotoxicity incidence and severity. Based on when it occurs, neurotoxicity can be characterised as either acute, subacute, or late. Seizures, confusion, somnolence, headaches, nausea, vomiting, and fever are only some of the acute neurotoxicity symptoms that can appear within hours of taking MTX ⁽¹⁷⁾.

Subacute MTx-induced encephalomyelitis might manifest as stroke-like symptoms such as hemiparesis, ataxia, speech difficulty, or myelopathy with sensory abnormalities, leg discomfort, and paraplegia days to weeks after treatment has begun. Learning impairments, neurocognitive impairment, and other leukoencephalopathic symptoms like quadriparesis, dementia, coma, and even death can occur months or years after the initial neurotoxic event in people with chronic neurotoxicity ⁽¹⁸⁾.

The development of neurotoxicity at high doses may be explained by biochemical processes associated with MTX action. When thymidine and purines aren't synthesized, DNA and RNA synthesis are affected. By blocking dihydrofolate reductase, MTX helps prevent folic acid breakdown (DHFR). Inhibition of purine synthesis and subsequent arrest in the S phase of the cell cycle ultimately lead to death of cells when dihydrofolate levels and tetrahydrofolates (THF), which is required for purine and thymidine synthesis, accumulate in the cell ⁽¹⁹⁾.

The overproduction of reactive oxygen species, the decrease in mitochondrial outer membrane potential, the swelling of mitochondria, the release of cytochrome c, and the increase in caspase-3 & 9, all leading to apoptosis, were all observed after MTX treatment, providing further evidence of MTX-induced oxidative stress. Moreover, the MTX-induced inflammation was characterised by an upregulation of NF-kappa B (NF-B) and IL-6, two pro-inflammatory proteins ⁽²⁰⁾.

Reduction in size, chromatin condensation, protein breakage, DNA breakdown, and phagocytosis are all morphological and biochemical changes associated with the apoptotic or programmed cell death

mechanism. Degenerative disorders of the central nervous system (CNS) such as Alzheimer's disease and Parkinson's disease, cancer, and immune system malfunction all have this factor in common ⁽²¹⁾.

Apoptosis is activated, regulated, and carried out by a plethora of components, the most of which are proteins. Too far, the Bcl-2 (B-cell lymphoma 2) family has been the best characterized protein family involved in the regulation of apoptotic cell death. This family includes both anti-apoptotic and pro-apoptotic members. Members of this family that are anti-apoptotic, like Bcl-2 and Bcl-x1 (B-cell lymphoma 2-extralarge), stop apoptosis from occurring in one of two ways: by sequestering proforms of death-driving cysteine proteases called caspases, or by blocking the release of cytochrome c and apoptosis-inducing factor (AIF) from mitochondria ⁽²²⁾.

When they reach the cytoplasm, cytochrome c and apoptosis-inducing factor (AIF) directly activate caspases, which cleave a series of cellular proteins to trigger apoptosis. On the other hand, pro-apoptotic members of this family like Bax and Bak induce caspase activation by releasing caspases from death antagonists through heterodimerization and by acting on the mitochondrial permeability transition pore to release apoptogenic substances. This means that the Bcl-2 family of proteins is an essential checkpoint in the pathways of apoptosis ⁽²³⁾.

Very little studies correlated the possible toxic effects of methotrexate on cerebellar cortex. **El-ghazouly et al.** ⁽²⁴⁾ aimed to assess the effects of methotrexate on the cerebellum of adult male albino rats on 60 male albino rates, The histological examination of methotrexate-treated group revealed marked destructive changes in Purkinje cells and most of cells of the molecular layer it was concluded that methotrexate caused marked histological, ultrastructural and immunohistochemical changes in the cerebellum of adult male albino rat indicating its neurotoxicity.

CONCLUSION

The mechanisms of MTX-induced neurotoxicity remain unclear yet as it has been less extensively investigated than other side effects of MTX. Purkinje cell shrinkage, karyolytic alterations in granule cells, and nuclear damage were observed as pathological changes at the cellular component of the cerebellar cortex after methotrexate exposure.

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REFERENCES

1. **Sasegbon A, Hamdy S (2021):** The role of the cerebellum in swallowing. *Dysphagia*, 21: 1-13.
2. **Mohanarajah S (2021):** Morphometric features of the adult cerebellum. *Neuroscience*, 104 (2): 499-512.

3. **Ajibade A, Ogundero S (2021):** Neurohistological changes in the cerebellar cortex of adult Wistar rats following lead acetate induced cortical damage. *GSC Biological and Pharmaceutical Sciences*, 15 (3): 182-188.
4. **Tam W, Wang X, Cheng A et al. (2021):** In search of molecular markers for cerebellar neurons. *International Journal of Molecular Sciences*, 22 (4): 1850-55.
5. **Alexander R, Bowie D (2021):** Intrinsic plasticity of cerebellar stellate cells is mediated by NMDA receptor regulation of voltage-gated Na⁺ channels. *The Journal of Physiology*, 599 (2): 647-665.
6. **Osorno T, Rudolph S, Nguyen T et al. (2022):** Candelabrum cells are ubiquitous cerebellar cortex interneurons with specialized circuit properties. *Nature Neuroscience*, 25 (6): 702-713.
7. **Rizza M, Locatelli F, Masoli S et al. (2021):** Stellate cell computational modeling predicts signal filtering in the molecular layer circuit of cerebellum. *Scientific Reports*, 11 (1): 1-17.
8. **Ransdell J, Nerbonne J (2018):** Voltage-gated sodium currents in cerebellar Purkinje neurons: functional and molecular diversity. *Cellular and Molecular Life Sciences*, 75 (19): 3495-3505.
9. **Fikry H, Saleh L, Abdel Gawad S (2022):** Neuroprotective effects of curcumin on the cerebellum in a rotenone-induced Parkinson's disease Model. *CNS Neuroscience & Therapeutics*, 28 (5): 732-748.
10. **Kim J, Augustine G (2021):** Molecular Layer Interneurons: Key Elements of Cerebellar Network Computation and Behavior. *Neuroscience*, 462: 22-35.
11. **Kozareva V, Martin C, Osorno T et al. (2021):** A transcriptomic atlas of mouse cerebellar cortex comprehensively defines cell types. *Nature*, 598 (7879): 214-219.
12. **Sotelo C (2017):** Cerebellar transplantation: a potential model to study repair and development of neurons and circuits in the cerebellum. In *Development of the Cerebellum from Molecular Aspects to Diseases*, ed. Marzban H. (Cham: Springer), Pp: 465-493. https://link.springer.com/chapter/10.1007/978-3-319-59749-2_22
13. **Brown A, Arancillo M, Lin T et al. (2019):** Molecular layer interneurons shape the spike activity of cerebellar Purkinje cells. *Sci Rep.*, 9: 1742-48.
14. **Younis N, Elsewedy H, Shehata T et al. (2021):** Geraniol averts methotrexate-induced acute kidney injury via keap1/nrf2/ho-1 and mapk/nf-kb pathways. *Current Issues in Molecular Biology*, 43 (3): 1741-1755.
15. **Famurewa A, Aja P, Maduagwuna E et al. (2017):** Antioxidant and anti-inflammatory effects of virgin coconut oil supplementation abrogate acute chemotherapy oxidative nephrotoxicity induced by anticancer drug methotrexate in rats. *Biomed Pharmacother.*, 96: 905-911.
16. **Maksimovic V, Pavlovic-Popovic Z, Vukmirovic S et al. (2020):** Molecular mechanism of action and pharmacokinetic properties of methotrexate. *Molecular Biology Reports*, 47 (6): 4699-4708.
17. **Al-Awwad A, Koriesh A (2021):** Cytotoxic Lesion in the Splenium of Corpus Callosum Secondary to Subacute Methotrexate Neurotoxicity. *Avicenna Journal of Medicine*, 11 (03): 160-162.
18. **Dabagh S, David H, Young S et al. (2020):** Severe, fatal methotrexate-related neurotoxicity in 2 adolescent patients with ALL. *Journal of Pediatric Hematology/Oncology*, 42 (8): 839-43.
19. **Pellacani C, Eleftheriou G (2020):** Neurotoxicity of antineoplastic drugs: Mechanisms, susceptibility, and neuroprotective strategies. *Advances in Medical Sciences*, 65 (2): 265-285.
20. **Ebrahimi R, Sepand M, Seyednejad S et al. (2019):** Ellagic acid reduces methotrexate-induced apoptosis and mitochondrial dysfunction via up-regulating Nrf2 expression and inhibiting the IκBα/NFκB in rats. *DARU Journal of Pharmaceutical Sciences*, 27 (2): 721-733.
21. **Nirmala G, Lopus M (2020):** Cell death mechanisms in eukaryotes. *Cell Biol Toxicol.*, 36: 145-164.
22. **Warren C, Wong-Brown M, Bowden N (2019):** BCL-2 family isoforms in apoptosis and cancer. *Cell Death & Disease*, 10 (3): 1-12.
23. **Singh A, Kukreti R, Saso L et al. (2019):** Oxidative stress: a key modulator in neurodegenerative diseases. *Molecules*, 24 (8): 1583.
24. **El-ghazouly D, Mahmoud B, Mansour M et al. (2021):** Histological and Immunohistochemical Study on the Effect of Methotrexate on the Cerebellum of Adult Male Albino Rats and The Possible Protective Role of *Lepidium Sativum*. *Egyptian Journal of Histology*, 44 (1): 241-255.