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Toxic Effects of Methotrexate on the Cerebellar Cortex of Adult Albino Rats and the Possible Protective Role of Vitamin C: An Electron Microscopic Study

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Abstract

Background: The cerebellum means the little brain which located behind the big brain. It was involvedin different functions like cognition, memory and motor activity. Methotrexate is anti-cancer drug widely used in chemotherapy regimens. Also, it was used as an anti-inflammatory drug. Methotrexate has serious side effects on the nervous system. Vitamin C is the most common antioxidant element; it acts as a free radical scavenger especially in the nervous tissue.

Aim of Study: The aim is to identify the serious toxic effect on the fine cellular structure of the cerebellar cortex of albino rats, and to determine if vitamin C can protect against serious of methotrexate or not.

Material and Methods: We used fifteen male albino rats and they were divided in to three groups. First group considered as control, the second received 10mg/kg by intraperitoneal injection as single dose weekly for four weeks. The third group received the same methotrexate dose and vitamin C as 20mg/kg through the intragastric tube every other day for four weeks. After one month of the experiments, rats were decapitated and brains were extracted and cerebella are separated and cut for different serial sagittal sections, they were processed for electron microscopic study.

Results: Exposure to methotrexate resulted in pathological changes at cellular component of cerebellar cortex in the form shrinkage of Purkinje cell, karyolitic changes in granule cells and nuclear damage was viewed and photographed by the electron microscope, but when vitamin C was administrated at the same time, these changes were decreased within Purkinje and granule cells.

Conclusion: Methotrexate is asharmful drug, it destroys the cortex oh cerebellum, but when vitamin C is administrated with it, it can diminish its harmful effect.

Key Words: *Cerebellar cortex – Methotrexate – Vitamin C.*

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Introduction

CEREBELLUM is the part of brain that involved in co-ordination of movement; also it provides correction of movements, motor learning and reflex modification **[1]**. Cerebellum is prompted be involved in multiple cognitive functions like language, spatial cognition and connected to many psychiatric conditions like autism, attention deficit hyperactivity disorders, mood disorders and schizophrenia **[2]**. Cerebellum lies in the posterior cranial fossabehind thepons and medulla oblongata from and it is separated from them by the fourth ventricle [3]. It is joined to brain stem by three bilaterally located peduncles; superior, middle and inferior [4]. It is covered superiorly by dural fold called tentorium cerebelli **[2]**. Cerebellum is formed of two main cerebellar hemispheres which are connected to each other by a vermis **[5]**, each cerebellar hemisphere is formed of outer gray matter called cortex and inner white matter **[6]**. Cerebellar cortex layers are molecular, Purkinje and granular cell layer arranged from outside to inside **[7]**. Methotrexate (MTX) is a folic acid antagonist and widely used in treatment of several neoplastic diseases such as osteosarcoma, acute lymphoblasticleukemia **[8]**. Also, it is used in treatment of non-neoplastic diseases like inflammatory or autoimmune disease **[9]**. MTX has multiple side effects especially on the nervous tissues **[10]**.

Vitamin C (VC) is one of the most common antioxidants **[11]**; it has the ability to protect cells from the reactive oxygen species (ROS) more than other antioxidants thus protect tissue from damage **[12]**.

Material and Methods

Fifteen adult male albino rats weighing 200-250gm, were obtained from the animal house of Sohag Faculty of Science. They were reared under standard conditions of feeding and temperature. Ethical clearance for the use of animals was got from the Institutional Animal Ethics Committee prior to the beginning of the work.

The animals were divided in to 3 groups:

- Group 1: Control group received no treatments.
- Group 2: Received 10mg/kg MTX throughintraperitoneal injection, it is injected once per week for four weeks **[13]**.
- Group 3: Received 20mg/kg VC through the intragastric tube every other day for four weeks **[14]** and methotrexate as the same dose at group 2.

After end of the experiment, animals were anaesthetized with ether then decapitated and skulls were opened, cerebella were taken and cut to sagittal sections then fixed immediately in 2.5% glutraldehyde, for 24 hours then processed according to **[15]**. Semi thin sections are stained by Toluidine blue 1%, ultrathin sections of 0.1um thickness are done from the selected areas and are examined by (Joel JEM 1010) electron microscope,these steps were processed at Faculty of Medicine, Sohag University at 2019.

Results

Group 1 (Figs. 1,2): Purkinje cell of control groupappears with large cell body, well defined nucleus and nuclear envelop. Cytoplasm is filled with mitochondria, rough endoplasmic reticulum (rER) and Golgi apparatus (Ga). Granule cell appears smaller in size than Purkinje cell; the nucleus is rounded with coarse central and peripheral chromatin, covered by thin shell of cytoplasm which is filled with ribosomes and rER. It is surrounded by myelinated axons of mossy fibers.

Group 2 (Figs. 3,4): Purkinje cell appears shrunken; its cytoplasm has multiple vacuoles lysosomes and inclusion bodies. Fragmented Golgi apparatus, mitochondria is dilated and swollen with destructed cristae, it has an irregular and ill-defined nuclear envelop and eccentric nucleolus with fragmented and condensed chromatin. Granule cell appears shrunken with fragmented faint nuclear chromatin (karyolitic changes), the cytoplasm is vacuolated and rarified with swollen mitochondria.

Group 3 (Figs. 5,6): Purkinje cell shows cytoplasm with multiple and normal shaped mitochondria and rER. Less identified vacuoles and lysosomes. Nuclear envelope is slight regular with blocks of condensed chromatin on the inner side. Granule cell appears large; its cytoplasm is hyperdense with multiple mitochondria. Nucleus has coarse chromatin with irregular nuclear envelop. Granule cell restored its normal regular shaped, nucleus has rounded and heterochromatic shape, but some granule cells still has faint chromatin.

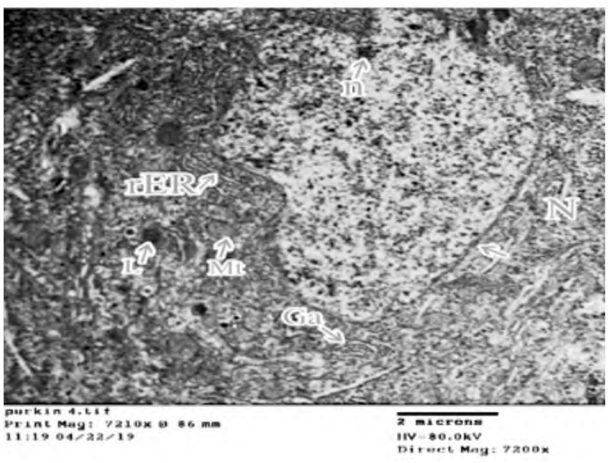

Fig. (1): An electron micrographof a section in the rat cerebellar cortex of control group of a Purkinje cell showing abundant mitochondria (Mt), rough endoplasmic reticulum (rER), Golgi apparatus (Ga), lysosomes (L), nuclear envelope (arrow), with eccentric nucleolus (n) (TEM x 7200).

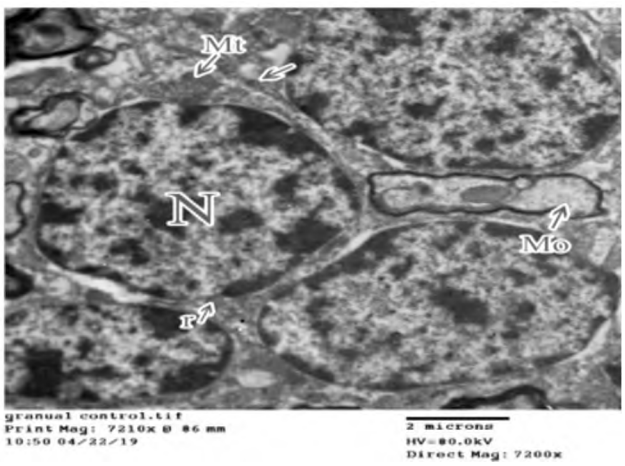

Fig. (2): An electron micrograph of a section in the rat cerebellar cortex of control group showing granule cell with well-defined nuclear envelop, large size heterochromatic nucleus (N), thin rim of cytoplasm (arrow) contain mitochondria (Mt) with normal crista, and free ribosomes (r), Mossy fibers (Mo) (TEM x 7200).

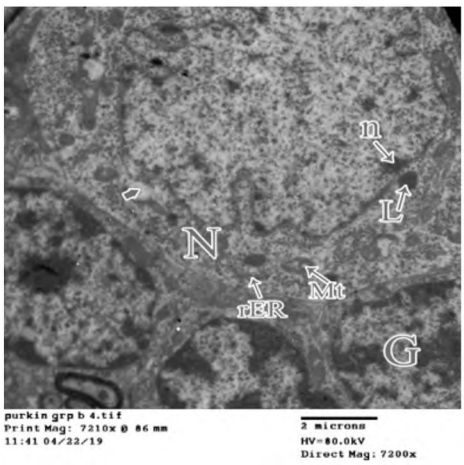

Fig. (3): An electron micrograph of a section in the rat cerebellar cortex of MTX treated group showing Purkinje cell with dilated rough endoplasmic reticulum (rER), swollen mitochondria (Mt) with destructed cristae. More abundant lysosomes (L), multiple vacuolated areasin cytoplasm (short arrow), Nucleus (N) with irregular nuclear envelop and fragmented chromatin, nucleolus (n) is eccentric, granule cell (G) (TEM x 7200).

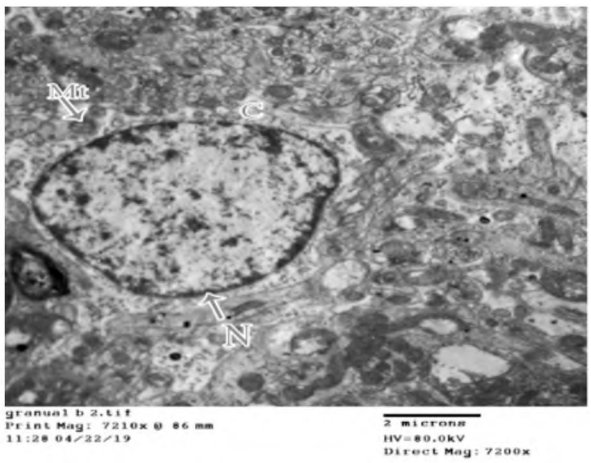

Fig. (4): An electron micrograph of a section in the rat cerebellar cortex of MTX treated group showing granule cell nucleus (N) with vacuolated and faint chromatin. Mitochondrion is swollen with destructed cristae (Mt), vacuolated rarified cytoplasm (C) (TEM x 7200).

Fig. (5): An electron micrograph of a section in the rat cerebellar cortex of MTX and VC treated group showing Purkinje cell with normal shaped mitochondria (Mt), endoplasmic reticulum is not dilated (rER), nuclear envelop is slightly regular and its inner aspect has blocks of chromatin (arrow), eccentric nucleolus (n), less identified lysosomes (L) and less vacuolated areas (arrow) (TEM x 7200).

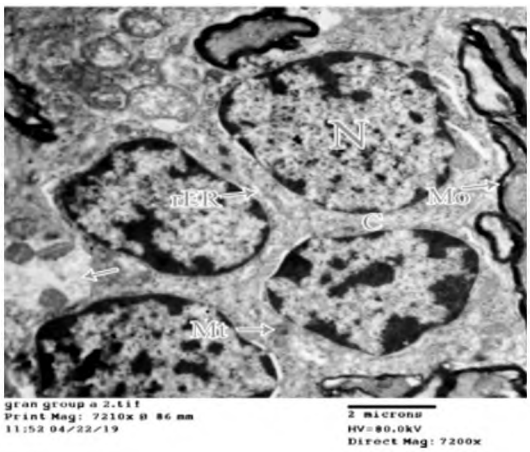

Fig. (6): An electron micrograph of a section in the rat cerebellar cortex of MTX an VC treated group showing granule cell with regular shape of cell and heterochromatic large nucleus (N), thin shell of cytoplasm (C) contains rough endoplasmic reticulum (rER) and mitochondria (Mt), mossy fiber axon is noticed (Mo). Vacuolated area is less present (arrow) (TEM x 7200).

Discussion

The cerebellum is an important organ for motor functins, cognition and emotion **[16]**, aspects of structural configuration, neuronal element, fiber comuication and neurotransmitters in crebellar cortex have been extensively investigated **[17]**. MTX is one of the most comon anti cancer drug due to its good results **[18]**. MTX has a serious neurological side effets like acute alteration of cosiousness, cerebral infacrctin, paralysisi, neuropathy and leuckoencepahalopathy **[19]**. VC is a water soluble vitamin that present in food and can protect body tissue from the free radicals as it is agood free radical scavenger **[20]**. In the present study, electron microscopic examination of MTX treated specimens showed Purkinje cell were reduced in size, increased density of the cytoplasm with vacuolated areas with multiple lysosomes, swollen destructed mitochondria and the nucleus appeared with irregular indented nuclear envelope.Granule cell showed shrinkage in size, faint nuclear chromatin, swollen mitochondria, these results were in the acceptance with **[13]** who found the same find-

ings in MTX injected guniea pigs. These ultrastrucurl changes are secondary to MTX oxidative stress effect in which there are intracellular biochemical events like inhibition of oxidtive phosphorylation processes within the Purkinje cells mitochondria, it leads to decreased phosphoipid synthesis and distrubted membrane integrity **[21]**. In the present study, electron microscopic examination of specimens received both VC and MTX as compared to MTX treated group that Purkinje cell restoredits normal shape, with slight regular nucleus, and normal shaped mitochondria, Purkinje cell contained euchromaticand near regular nucleus with prominent nucleolus. Granular cell had rounded oval nuclei with round heterochromatic nucleus, some granule cells appeared similar to those in the control group. These results were in acceptance with **[22,23]**. Anti-oxidants are substances that may defend the body cells against the impacts of free radicals provoked by oxidative damage in different cellular compartments and tissues **[22]**. On the other hand, cerebellar white matter in treated rat with lead and VC showed histological changes just like normal **[14]**. Also, VC protect cerebellum from oxidative stress effect following exposure to radiofrequency **[24]**.

Conclusion:

Methotrexate is a highly toxic drug to cerebellar cortex; it destructs the fine structure of Purkinje cells and granule cells while administration of vitamin C at the same time can diminish this toxicity.

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التأثيرات السامة للميثوتريكيست على التركيب النسيجى لقشرة مخيخ الفئران البيضاء البالغة والدور الوقائى المحتمل لفيتامين ج؛ دراسة بالمجهر الالكترونى

الميثوتريكست هو الأكثر إستخداماً فى الأ مراض المناعية والأورام والإلتهابات على مدى واسع من الجرعات ويؤثر على الجهاز العصبى والخلايا العصبية بشكل خاص فهو ناجم عن زيادة الأكسدة للخلايا والأغشية الخلوية الخاصة بها. فيتامين ج ٥ يذوب فى الماء ولكنه لا يصنع فى الجسم وهو أهم مضادات الأكسدة ويساهم فى حماية خلايا الجسم من العناصر الطيارة وهو يحمى جزيئات الدهون والبروتين فى الخلايا العصبية وقد قمنا بعمل دراسة نسيجية للتأثير السام للميتوتريكسيت على طبقات القشرة الخاصة بالمخيخ والتأكد من وجود دور و قائى محتمل لفيتامين ج يضاد تأثير الميثوتريكسيت وذلك بالفحص النسيجى بالمجهر الالكترونى. واستخد منا خمسة وأربعون فأر بالغ فى هذه الدراسة حيث قسمت الفئرن إلى ثلاث مجموعات بطريقة عشوائية، كل مجموعة تحتوى على ١٥ فأر. المجموعة الأولى (مجموعة ضابطة)، المجموعة الثانية (مجموعة الميثوتريكسيت) وقد تم إعطائها جرعة ١٠ ميللجرام لكل كيلو جرام من وزن الفأر كل أسبوع لمدة ٤ أسابيع، أما المجموعة الثالثة (مجموعة الميثوتريكسيت وفيتامين ج) فقد تلقت نوعين: الأول وهو الميثوتريكسيت بنفس الجرعة السابقة والثانى وهو فيتامين ج بجرعة ٢٠ ميللجرام لكل كيلو جرام من وزن الفأر وتعطى الجرعة عن طريق أنبوب للفم والذى يصل إلى المعدة مباشرة والجرعة تقسم يوم ويوم وتستمر التجربة لمدة ٤ أسابيع، ثم تحضير العينات بالطرق المعتادة تم فحص عينات المجموعة الضابطة. تم فحص عينات المجموعة الثانية والثالثة لمقارنتها بالمجموعة الضابطة وقد بين الفحص للمجموعة الثانية أن خلية بركنجى أصغر من حجمها المعتاد وفى السيتوبلازم حيث ظهرت الميتوكوندريا منتفخة وممزقة وظهر بداخله فراغات ڤيسوزومات، أما النواة فقد فقدت إنتظام الغشاء النووى بالنسبة للخلايا الحبيبية فقد ظهرت بغشاء نووى رقيق وقل حجم الكروماتين بداخل النواة داخلها، أما السيتوبلازم فقد بعض العضيات والميتو كوندريا انتفخت وتمزقت وعند فحص المجموعة الثالثة وجد إستعادة خلية بركنجى والخلايا الحبيبية للشكل الطبيعى والتركيب الطبيعى تقريباً.