Original Article

The Potential Ameliorative Effect of Vitamin C Versus Astaxanthin on Methotrexate Induced Damage in Rat Cerebellar Cortex: A Biochemical, Histological and Immunohistochemical Study

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ABSTRACT

Introduction: Methotrexate causes injury in the cerebellar cortex. Vitamin C is one of the most familiar antioxidant components. Astaxanthin may provide protection for the brain from reactive stress. The purpose of this study was to compare between the possible protective roles of vitamin C and Astaxanthin on cerebellar cortex.

Material and Methods: Thirty-six adult male albino rats were used in the study equally divided into a control group, methotrexate treated group, methotrexate + vitamin C group and methotrexate + astaxanthine group. By the end of the tenth day of the experiment, the cerebellar hemisphere had been obtained. The left cerebellar hemispheres were prepared for biochemical examination to evaluate the levels of MDA, CAT, GSH and SOD in cerebellar tissue. The right cerebellar hemispheres were prepared for histopathological examination by H& E and immunohistochemical examination by caspase-3 and GFAP immune-staining.

Results: Methotrexate (MTX) significantly elevates MDA level and significantly decrease the level of SOD, CAT and GSH in cerebellar tissue. MTX causes destruction of the histological structures of cerebellar cortex and induce significant elevation in caspase-3 and GFAP immunoreactivity in compared with control group. Vitamin C and astaxanthin (AXA) significantly ameliorate the biochemical and histopathological changes induced by MTX. AXA significantly decrease MDA level and significantly elevate SOD, CAT & GSH levels in compared to MTX+ vitamin C group. AXA preserve the normal histological structures of cerebellar cortex and significantly decrease caspase-3 and GFAP immuonreactivity in compared to MTX + vitamin C group.

Conclusion: Both vitamin C and astaxanthin have ameliorative roles in methotrexate induced cerebellar cortex injury. However, astaxanthin is better than vitamin C in protection against cerebellar cortex damage.

Received: 28 February 2023, **Accepted:** 30 April 2023

Key Words: Astaxanthin, caspase-3, GFAP, methotrexate, vitamin c.

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ISSN: 1110-0559, Vol. 47, No. 2

INTRODUCTION

Methotrexate is an efficient cytotoxic agent and folic acid antagonist; it inhibits cellular proliferation. It is used in management of different types of malignant tumors, also it is anti-proliferative and cytotoxic medication used to treat autoimmune conditions including rheumatoid arthritis, and inflammatory diseases[1]. Methotrexate crosses the blood-brain barrier (BBB), it has a damaging effect on the nervous tissue, by creating oxygen reactive species, decreases the amounts of antioxidant enzymes while causing oxidative stress, leading to apoptosis, tissue damage and causing neurotoxicity[2].

High doses of methotrexate result in adverse effects on central nervous system as cognitive damage, white matter demyelination and reduction of hippocampal neurogenesis[3,4]. Acute MTX toxicity on CNS may causes seizures, aphasia, hemiparesis, diplopia, dysphagia, and dysarthria. Chronic MTX toxicity may result in behavioral changes[5].

Vitamin C is present in nervous tissue in a high level than other organs. It has important role in appropriate functions of nervous system and it participates in the antioxidant defense in brain. It plays essential role in neuronal maturation and neurotransmission[6].

Rats exposed to neurotoxic chemicals, vitamin C therapy has been shown to reduce neuropathological changes, memory deficits, and neurodegenerative changes[7].

Astaxanthin (AXA) is a xanthophyll carotenoid, it is present naturally in seafood such as salmon, microalgae and crayfish, it is also available commercially[8]. AXA has an antioxidant, immunomodulatory, anti-apoptotic and anti-inflammatory properties[9]. AXA easily cross the blood-brain barrier; it is able to diminish lipid oxidation and neuronal losses by reducing oxidative injure[10]. AXA

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can induce protective mechanism in neurodegenerative, oxidative stress, metabolic disorders and inflammatory diseases[11].

The current study prepared to compare between the possible protective roles of vitamin C and Astaxanthin on cerebellar cortex.

MATERIALS AND METHODS

Animals

36adult male albino rats, weighting about200- 220 g, they were obtained from faculty of veterinary medicine, Benha University. All rats were kept at temperature (24°C) and humidity (60%). They were nourished normal food and free admission for water. Rats were housed for one week preceding the beginning of the study. The experiment was accepted by the Ethical Committee of Benha University number (RC- 14-11-2022), date of acceptance 10/1/2023.

Drugs

Methotrexate; available as methotrexate 2 ml vial, 25 mg/ml injection. It was obtained from Mylan Pharmaceutical Company.1ml of MTX contained 25mg of MTX, that was diluted in 3 ml saline each ml contained 5mg of MTX.

Vitamin C; available as C Retard Ascorbic acid 500mg capsules, it was obtained from Hikma Pharmaceuticals Company. Each capsule was dissolved in 10 ml distilled water, each 1ml contained 50mg vitamin C.

Astaxanthin; available as astaxanthin 4mg, 30 soft gels capsules by Biovea Company. Each soft gel capsule was aspirated and dissolved in 1ml of olive oil.

Groups

All rats were separated equally into four groups each including nine rats, for a period of ten days.

Group I: Control group; divided equally into 3 subgroups

- Subgroup (Ia); rats were kept without any medications.
- Subgroup (Ib); rats were given 250 mg/Kg body weight of Vit C by oro-gastric tube for ten days.
- Subgroup (I c); rats were given 20 mg/kg body weight of AXA by oro-gastric tube for ten days.

Group II: MTX group; rats were given MTX 20 mg/kg body weight single i.p. injection at the fifth day of the experiment[12].

Group III: MTX + Vit C group; rats were given 250 mg/Kg body weight of Vit C by oro-gastric tube from the first to the tenth day and MTX single i.p. injection in the fifth day as in group II one hour after the dose of vit C at this day[13].

Group IV: MTX + AXA group, rats were given 20 mg/kg body weight of AXA by oro-gastric tube from the first to the tenth day and MTX single i.p. injection in the fifth day as in group II one hour after the dose of AXA at this day[14].

By the end of the tenth day all rats were anaesthetized by intraperitoneal injections of Ketamine hydrochloride. After that, vault of the skull was removed; brain was dissected out in each rat. Cerebellum hemispheres were dissected carefully and separated sagittally into two halves, then the right hemisphere rapidly fixed in formalin for 2 days for histological examination. The left side of the cerebellum was prepared for biochemical analysis.

Biochemical examination

Tissue specimens were prepared for biochemical analysis by frozen in a potassium phosphate buffer that is extremely cold and minced at 20 °C in Scientific Research Centre, Faculty of Medicine, Benha University. Centrifuging the mixture for half an hour, and the resultant supernatant was used to evaluate the malondialdehyde (MDA) level according to[15]. Determination of tissue antioxidant Enzyme Activity as superoxide dismutase (SOD)[16], catalase (CAT)[17] and, reduced glutathione (GSH)[18], were analyzed.

Histological examination

The right half of cerebellar hemisphere samples were put in 10 % formalin solution, then handled for light microscopic examination protocol including dehydration with alcohol, cleared by xylene after that they were fixed in paraffin then sectioned at 4-5 µm thickness for successive histopathological and immunohistochemical examination. Staining with hematoxylin and eosin (H&E) for routine histological analysis[19].

Immunohistochemical study

Caspase-3 immunostaining: used for demonstration of cell apoptosis. The de-paraffinized 5 µm thickness paraffin sections which were put on positively charged slides were incubated in 10% hydrogen peroxide in absolute methanol (10 min). Heating the sections in 0.01 mol/l citrate buffer (pH: 6) in a water bath in a microwave (30 min) was done to unmask performed antigen. The slides were rinsed for 5 min in PBS at pH 7.4 and the sections were incubated with primary antibody (1h). The sections were treated with caspase-3 antibody at appropriate dilution in antibody dilution buffer in a humidified chamber (1 h) at room temperature, then were washed twice with PBS-T (5 min), to remove excess chromogen. The sections were incubated with the secondary antibody (20 min), Then washed with PBS for 3times (5min). DAB was added (10 min). Finally, the slides were counterstained by Mayer's hematoxylin, dehydrated, cleared, and mounted by DPX. Cytoplasmic brown coloration was considered positive reaction[20].

(GFAP) Glial fibrillary acidic protein: used for recognition of neuroglial astrocytes. Paraffin sections were incubated for 10 minutes in 10% hydrogen peroxide. Slices were then heated in a microwave to reveal the performed

antigen in 0.01 mol/l citrate buffer in a water bath for half an hour, after that they were rinsed in PBS at pH 7.4 after that they treated with primary antibody anti GFAP (Ab-1, clone GA-5 mouse monoclonal antibody for one hour[21]. The secondary antibody was added at 25C for one hour. then incubated for ten minutes in preformed streptavidin peroxidase. After that they were incubated in substrate chromogen3,3'- Diaminobenzidine for 15 minutes that causes a brown-colored precipitate at the antigen sites, then counterstained by Harris's hematoxylin to stain nuclei. The main antibody was substituted with non-immune rabbit serum in the negative control sections. The positive control sections in brain (cerebellum and striatum)[22].

Morphometric measurements

Computer assisted digital image analysis. Slides (Ten non overlapping fields from each groups) that photographed using Olympus ® digital camera installed on Olympus ®microscope with 0.5 X photo adaptor, using 40 X objective and saved as TIFF. The result images were analyzed on Intel ® Core I7 ® based computer using Video Test Morphology ® software (Russia) with a specific built-in routine for Caspase-3 % area & GFAP % area. Results were exported to Excel Sheet for Statistical analysis[23].

Statistical analysis

By using the (SPSS) version 20 to analyze the acquired data (SPSS Inc., Chicago, Illinois, USA). *P value* was regarded highly significant if *P value* was 0.01, significant if *P value* was 0.05, and non-significant if *P value* was> 0.05. Every piece of data was reported as mean SD.

RESULTS

Biochemical results

When compared to the control group (12.281.29 nmol/g), MDA values were significant increase in the MTX-treated group (29.331.86 nmol/g). Administration of Vit C plus MTX in group III significantly reduce the level of MDA to be (19.08±2.81 nmol/g). AXA also significantly decreased MDA level in group IV to be (15.93±1.45 nmol/g) in comparison to the MTX treated group. Moreover, significant reduction in MDA level in group IV (15.93±1.45 nmol/g) in comparison with group III (19.08±2.81 nmol/g).

SOD, CAT and GSH levels were decreased significantly in group II (2.11±0.41 u/g, 0.72±0.15 u/g, and 1.03±0.14 nmol/g) in comparison to group I(4.46±0.46 u/g,1.86±0.20 u/g and 2.51±0.28nmol/g). The levels of SOD, CAT and GSH in group III (2.62±0.27 u/g, 0.99±0.11 u/g and 1.83±0.19nmol/g) in that order were significantly higher than that in group II. but still was significantly difference in compared to group I. In group IV the antioxidant enzymes were (3.90±0.21 u/g, 1.29±0.19 u/g and 2.26±0.26 nmol/g) correspondingly were significantly elevated than those in group II and significant higher than group III. (Table 1, Histograms 1,2,3,4).

Histological Results

Cerebellar sections stained by H&E stain

Cerebellar cortex sections in the control rats in subgroups (I a, I b& I c) were identical and showed the outer molecular, middle Purkinje, and inner granular layers of cerebellar cortex. The molecular layer shows normal basket cells, with lightly acidophilic neuropil. The Purkinje cells were large, pyriform in shape with long dendrites toward the molecular layer. They have basophilic granular cytoplasm, central nuclei and prominent nucleoli, they arranged in one raw. The granular layer consisted of granular cells with deeply stained nuclei, there was lightly stained acidophilic glomeruli among the granular cells (Figure 1).

Sections from group II (MTX group) showed loss of normal histological structures of cerebellum as the molecular layer showed basket cells with pyknotic nuclei. Purkinje cells appear irregular shaped, shrunken and deeply stained pyknotic nuclei, with lose their dendrites and surrounded by vacuolated neuropil. Distorted irregular shaped purkinje cell among cells of granular layer. Other purkinje cells show vacuolated cytoplasm with loss of its nucleus. Granular layer showed dispersed, shrunken, deeply stained and irregular shaped granular cells and closely aggregated granular cells. The cerebellar glomeruli showed loss of normal architecture (Figure 2).

Sections from group III (MTX + Vit C group) the molecular layer showed normal basket cells and others with pyknotic nuclei. The purkinje cells are dispersed some appeared with normal shape. Other purkinje cells were irregular in shape, shrunken with pyknotic nuclei and vacuolated neuropil. The granular layer has some clumped darkly stained granule cells. The cerebellar glomeruli showed loss of normal architecture (Figure 3).

Sections from group IV (MTX + AXA group) showed normal molecular layer showed apparently normal basket cells, few with pyknotic nuclei, the purkinje layer have normal pyriform in shape purkinje cells. There were degenerated and vacuolated areas between the purkinje cells. The granular cell layer has normal granule cells (Figure 4).

Caspase-3 Immunohistochemical stain

Cerebellar cortex section from group I showed negative caspase-3 immunoreaction in molecular, Purkinje and granular cell layers (Figure 5a). Group II revealed high positive caspase-3immunoreaction in all layers (Figure 5b). Group III showed moderate positive caspase-3 immunoreaction (Figure 5c). Sections from Group IV demonstrated few positive caspase-3 immunoreaction (Figure 5d).

GFAP Immunohistochemical stain

GFAP immunohistochemical staining of cerebellar cortex from the control group presented few number of astrocytes with positive GFAP immunoreaction.

(Figure 6a). Rats from MTX treated group represented large number of astrocytes with positive GFAP immunoreactions (Figure 6b). MTX + Vit C treated group revealed moderate staining intensity of astrocytes cells with positive GFAP immunoreaction in cerebellar cortical layers (Figure 6c). Rats from MTX + AXA treated group presented few astrocytes with GFAP positive immunoreaction in all layers (Figure 6d).

Morphometric results

Caspase-3 immunostaining

The mean area percentage (%) of Caspase-3 positive immunoreaction was significantly increased in the MTX treated group as compared to the control group. It was significantly decreased in the MTX + Vit C and MTX + AXA treated groups as compared to the MTX treated group. Moreover, the mean area percentage (%) of Caspase-3 positive immunoreaction in MTX + AXA treated group was significantly decreased as compared with MTX + Vit C treated group. As represented in (Table 2, Histogram 5)

GFAP immunostaining

The mean area percentage (%) of GFAP positive immunoreaction was significantly increased in the MTX treated group as compared to the control group. It was significantly decreased in MTX + Vit C and MTX + AXA treated groups as compared to the MTX treated group. But we have to notice that the mean area percentage (%) of GFAP positive immunoreaction in MTX + AXA treated group was significantly lower in compared with MTX + Vit C treated group. As represented in (Table 2, Histogram6)

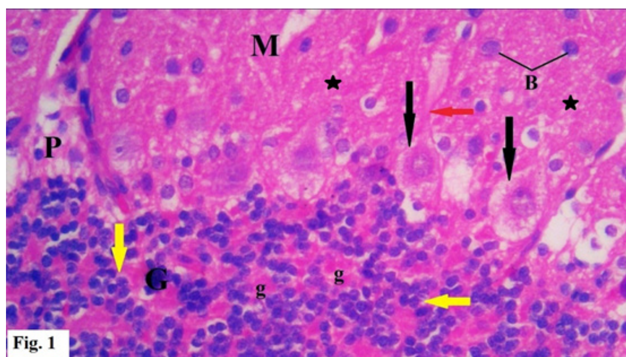


Fig. 1: A photomicrograph of cerebellar cortex from group I, showing (M) the molecular layer has normal basket cells (B) with lightly acidophilic neuropil (stars). Purkinje cell layer (P) showing large purkinje cell (black arrows) pyriform in shape with long dendrites toward the molecular layer (red arrow). They have basophilic granular cytoplasm, central nuclei and prominent nucleoli they arranged in one raw. Granule cells (yellow arrows) with deeply stained nuclei at the granular layer (G) there was lightly stained acidophilic glomeruli (g) among the granular cells (H&EX400)

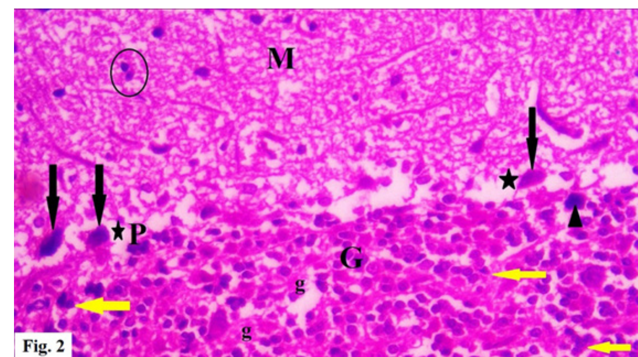


Fig. 2: A photomicrograph of cerebellar cortex from group II, showing the molecular layer (M) has basket cells with pyknotic nuclei (circle). Purkinje cells (P) appear irregular shaped, shrunken with deeply stained pyknotic nuclei (black arrows), with lose their dendrites and surrounded by vacuolated neuropil (stars). Irregular indistinct purkinje cell (head arrow). Other purkinje cells show vacuolated cytoplasm with loss of its nucleus (area between two stars). Granular layer (G) showing dispersed, shrunken, deeply stained and irregular shaped granule cells and closely aggregated granular cells (yellow arrows). The cerebellar glomeruli show loss of normal architecture (g). (H&EX400)

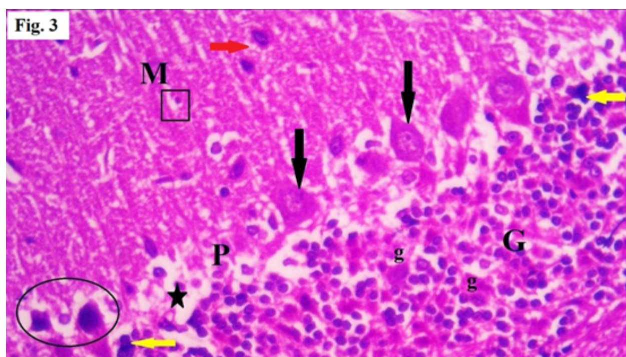


Fig. 3: A photomicrograph of cerebellar cortex from group III, showing the molecular layer (M) has normal basket cells (red arrow), and others with pyknotic nuclei (sqaure). At purkinje cell layer (P) the purkinje cells are dispersed, some appeared with normal shape (black arrows). Other purkinje cells (circle) were irregular in shape, shrunken with pyknotic nuclei and vacuolated neuropil (stars). The granular layer (G) has some clumped darkly stained granule cells (yellow arrows). The cerebellar glomeruli show loss of normal architecture (g). (H&E X400)

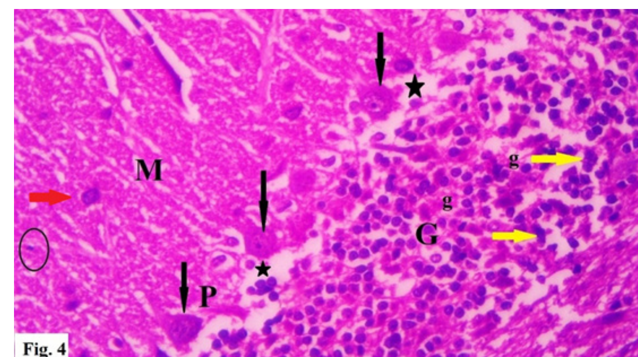


Fig. 4: A photomicrograph of cerebellar cortex from group IV, viewing normal molecular layer (M) has normal basket cell (red arrow) and few with pyknotic nuclei (circle), purkinje layer (P) has normal pyriform in shape purkinje cells (black arrows). Notice degenerated and vacuolated areas (star). Granular cell layer (G) show normal granule cells (yellow arrows).

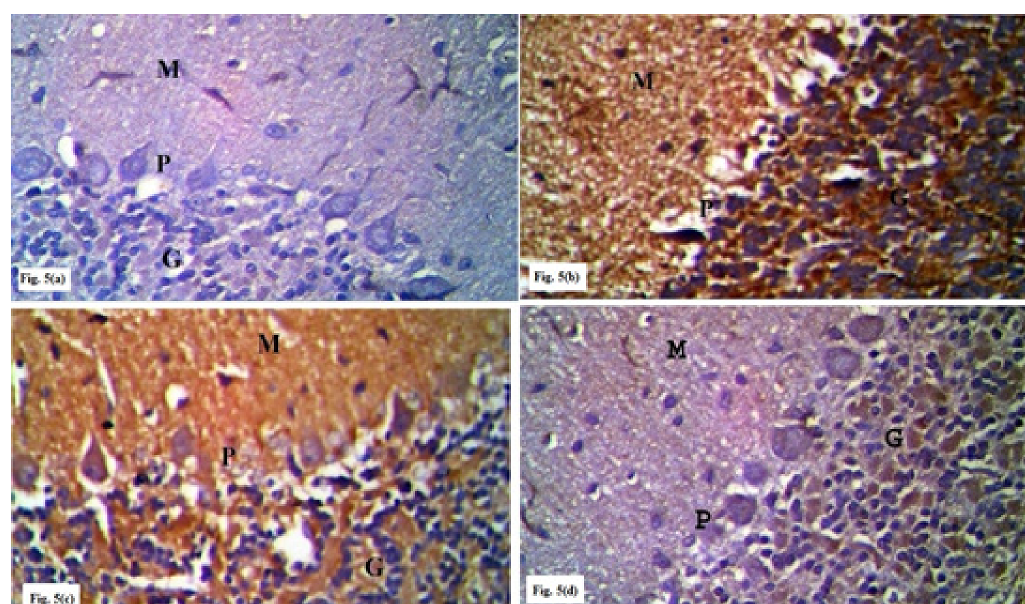


Fig. 5: Photomicrographs of cerebellar cortex sections (a): Group I showing negative caspase-3 immunoreaction in molecular, Purkinje and granular cell layers (b): Group II showing highly positive caspase-3 immunoreaction in all layers. (c): Group III showing moderate positive caspase-3 immunoreaction. (d): Group IV showing few positive caspase-3 immunoreaction. Molecular layer (M) Purkinje layer (P) and granular layer (G) (Caspase-3 immunostaining X 400)

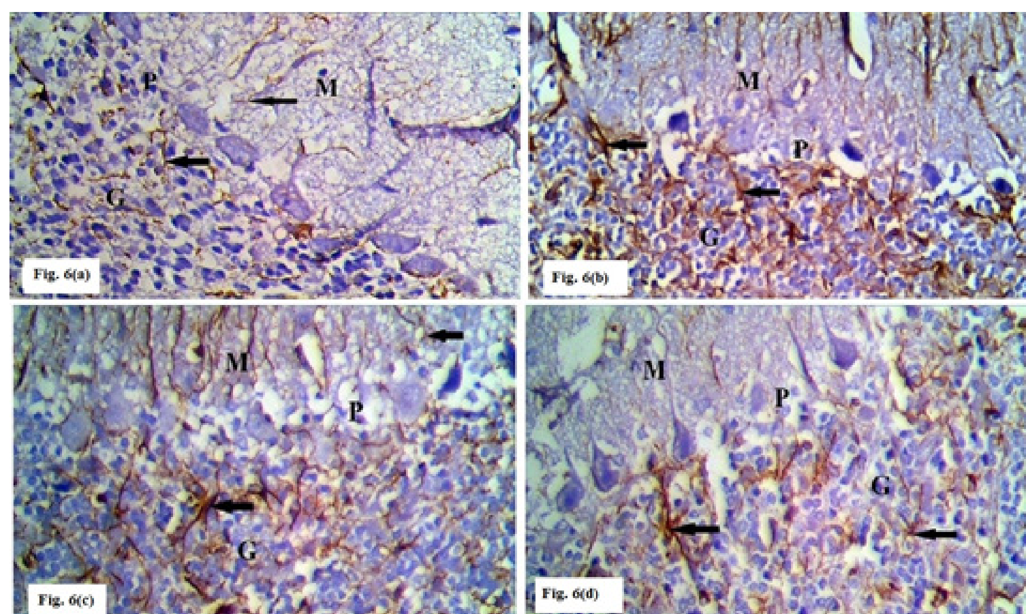


Fig. 6: Photomicrographs of cerebellar cortex sections (a): Group I showing few number of GFAP positive astrocytes (arrows). (b): Group II showing large number of GFAP positive astrocytes (arrows). (c): Group III showing moderate number of GFAP positive astrocytes (arrows). (d): Group IV showing few GFAP positive astrocytes (arrows). Molecular layer (M) Purkinje layer (P) and granular layer (G). (GFAP immunostaining

Table 1: Comparison of MDA (nmol/ g), SOD (u/g) , CAT (u/ g) & GSH (nmol/ g) levels between the studied groups.

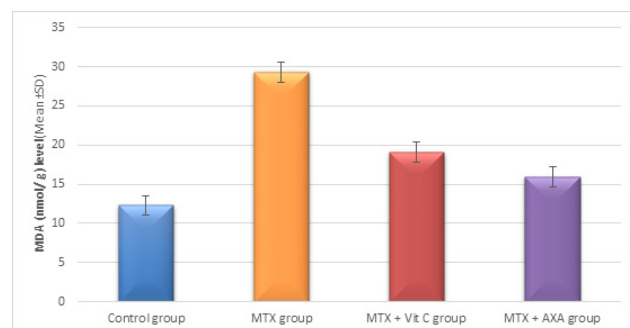
	Control group	MTX group	MTX + Vit C group	MTX + AXA group
MDA (nmol/ g)	12.28±1.29	29.33±1.86 ^{a}	19.08±2.81 ^{ab}	15.93±1.45 ^{abc}
SOD (u/g) level	4.46±0.46	2.11±0.41^{a}	2.62±0.27 ^{ab}	3.90±0.21 ^{abc}
CAT (u/ g) level	1.86±0.20	0.72±0.15 ^{a}	0.99±0.11 ^{ab}	1.29±0.19 ^{abc}
GSH (nmol/ g)	2.51±0.28	1.03±0.14 ^{a}	1.83±0.19 ^{ab}	2.26±0.26 ^{bc}

Data expressed as Mean± SD. *:significance <0.05 a: significance vs control group, b: significance vs MTX group, c: significance vs MTX + Vit C group

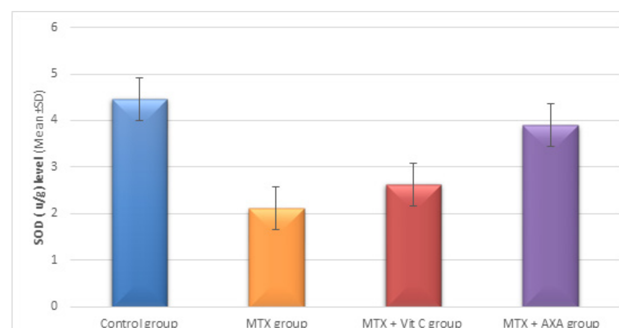
Table 2: Comparison of Caspase-3 % area & GFAP % area between the studied groups.

	Control group	MTX group	MTX + Vit C group	MTX + AXA group
Caspase-3 % area	1.86±0.64	70.22±9.10[a]	46.70±6.44[ab]	11.85±1.75[abc]
GFAP % area	3.04±0.67	18.59±2.16[a]	11.32±1.51[ab]	7.72±1.31[abc]

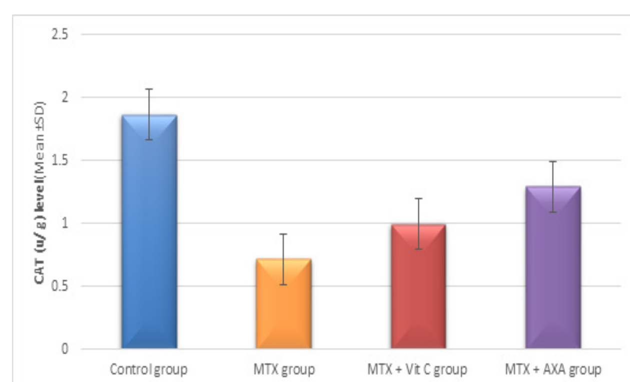
Data expressed as Mean±SD *: significance <0.05 a: significance vs control group, b: significance vs MTX group,c: significance vs MTX + Vit C group



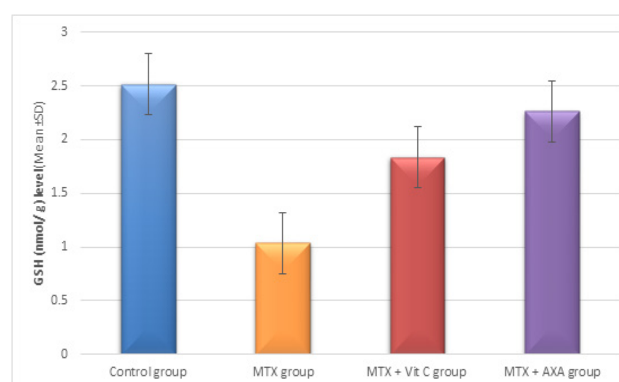
Histogram 1: Mean± SD of MDA (nmol/ g) level in studied groups



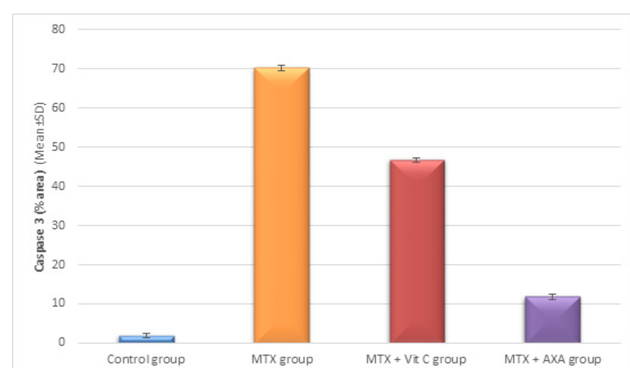
Histogram 2: Mean± SD of SOD (u/g) level in studied groups



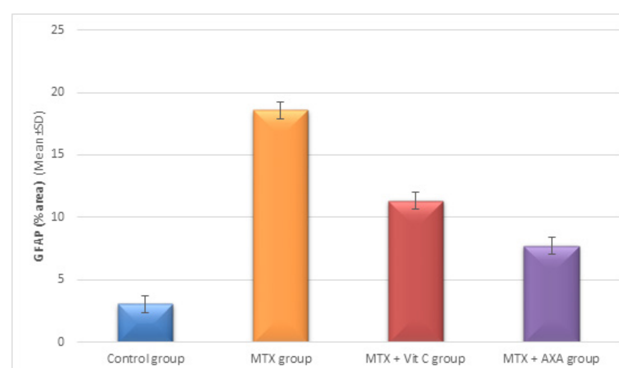
Histogram 3: Mean± SD of CAT (u/ g) level in studied groups



Histogram 4: Mean± SD of GSH (nmol/g) level in studied groups



Histogram 5: Mean ± SD of Caspase-3 % area in studied groups



Histogram 6: Mean ± SD of GFAP % area in studied groups

DISCUSSION

The cerebellum has a major role in regulation of the movement by controlling the muscle tone and the balance[24]. The cerebellum concerned in numerous cognitive functions as awareness, education, memory and sensory discrimination[25].

MTX induced neurotoxicity through impairment of mitochondrial functions and releases excessive reactive oxygen species that may lead to cell apoptosis. MTX neurotoxicity might occur by prolonged low-dose or single high dose of oral MTX administration[26].

Vitamin C has been concerned in development and maturation of neuron and glial cells[27]. AXA has anti-oxidant, anti-apoptotic, immunomodulatory and anti-inflammatory effects[28]. It can reduce oxidative damage by decreasing neuronal loss and lipid per-oxidation[29].

This study was done to compare between the possible protective roles of vitamin C and Astaxanthin on cerebellar cortex.

In this study the biochemical examination of group II revealed a significant elevation in MDA level in compared to group I. Antioxidant enzymes activity SOD, CAT and GSH levels were significantly reduced in MTX-treated group in comparison with that in control group indicating the role of MTX in impairment of biochemical analysis in cerebellar tissue and inducing oxidative stress. This result was in agreement with[26] who found that MTX enhanced oxidative stress in brain tissue due to the imbalance between antioxidants and oxidants, that lead to deterioration in the cellular functions and might cause different pathological conditions. The high level of MDA in brain tissue decreased the neurotransmitter- receptor interaction and membrane permeability[30].

Another study described the mechanism of damaging effect of MTX was by oxidative stress, reduction in cell proliferation, damage of the DNA, cytokine activation and encephalopathy[31]. Some authors stated that oxidative stress stimulated the mitochondrial apoptotic pathways[32].

In this study MTX induced damage in the normal histological structures of cerebellum tissue as there was shrunken irregular Purkinje cells, with deeply stained pyknotic nuclei with lose their process and surrounded by vacuolated neuropil. Other purkinje cells showed complete degeneration with vacuolated cytoplasm. Moreover, cells in the granule layer were dispersed, shrunken and severely stained. The main area percentage of caspase-3 and GFAP immunoreactions were significant increase in compared to the control group. Similarly,[33] stated that MTX causes destructive in the normal histological structures of cerebellar cortex with noticeable decline in the Nissl's granules in pyrkinje cells and they found that Purkinje cell, granular cell appeared degenerated with numerous cytoplasmic vacuoles, empty spaces by electron microscopic examination. Other authors explained the elevation in GFAP intensity to the extreme production of astrocyte (gliosis) adjoining with Purkinje cells apoptosis[34]. Cerebellar injury induced gliosis as a protective mechanism following neuronal degeneration[35].

Another study[36] revealed delayed cerebellum development in infant rats and explained these changes to neurotoxin effect of MTX in cell proliferation activity and cell apoptosis. Other authors revealed that Methotrexate induced highly positive expression of GFAP and Caspase-3 that indicates tissue damage and apoptosis in cerebellum, hippocampus, liver and kidney[37].

In this study vitamin C administration showed significantly decrease MDA level and significantly elevated the antioxidant enzymes activity SOD, CAT and GSH levels when comparison with the MTX group and these results were supported by other authors[38] who stated that administration of Vit C ameliorated the antioxidant system and decreased the oxidative stress caused by MTX. This was explained by[39] who proved that vitamins C able to augment the anticancer effects of little dose methotrexate. MTX plus vitamin C prevent the human glioblastoma multiform cell growth through the caspase-3 death pathway. Vit C diminishing the cell injures through reacting with oxygen superoxide and hydroxyl radicals[40].

In this study vitamin C mild prevented the pathological changes of MTX on cerebellar tissue as the molecular layer has normal basket cells and others with pyknotic nuclei. The purkinje cells are dispersed some appeared with normal shape. Other purkinje cells were irregular in shape, shrunken with pyknotic nuclei and vacuolated neuropil. The granular layer has some clumped darkly stained granule cells. These results were established by a significant decrease in the main area percentage of caspase 3 and GFAP immunoreaction when compared with those in MTX group. Our findings were in accordance with[41] who revealed that MTX is very toxic to cerebellar cortex, but vitamin C conserved the normal thickness of molecular layer that appeared has more cellularity. Purkinje cells has normal size and arrangement, while others appeared irregular with less vacuolated areas around it, with normal distributed granular cell.

Another study can confirm our results about the protective role of Vit C by their electron transmission results as they found that MTX destroy the ultra-structures of Purkinje and granule cells. Although, vitamin C could diminish the neurotoxicity of MTX, as the Purkinje cell restored its normal ultra-structures[42]. Also,[43] stated the capacity of vitamin C to diminish the oxidative stress status caused by MTX in renal and hepatic tissue. Vitamin C ameliorated the neurotoxicity induced by gibberellic acid in pregnancy and lactation on off springs' cerebellar cortex in albino rats[44].

In the current study AXA administration was significantly decrease MDA level and significantly elevated the levels of SOD, CAT and GTH in compared to the MTX group and MTX+ Vit C treated group indicating the powerful efficacy of AXA in prevention of MTX neurotoxicity than vitamin C. In the same line[45] reported

that AXA ameliorated the elevation of Myelin basic protein expression and decreased the activity of Growth related oncogene, Granulocyte colony-stimulating factor levels that elevated by MTX.

Another study added that AXA controlled the inflammatory cytokine level and ameliorated the oxidative stress that causes impairment in spatial memory and hippocampal neurogenesis[46]. AXA attenuated MDA level and increased the antioxidant enzyme level with improvement in the histopathological changes in brain tissue. AXA could be used for protecting the brain tissue from cadmium neurotoxicity[14].

In the present study AXA administration markedly improved the histopathological changes caused by MTX as the histological structures of cerebellum appeared nearly normal, these findings confirmed by significant decrease in the main area percentage of caspase-3 and GFAP immunoreactions in compared with those in MTX group and MTX+ vitamin C groups. These findings stated that AXA has a high protective role than vitamin C in prevention of MTX neurotoxicity on cerebellum.

These results were in agreement with[47] who detected the protective effect of AXA in the edema and neuronal degeneration in the hippocampus and cerebral cortex produced by MTX. Also pretreatment of AXA could protect germ cells from oxidative stress produced by MTX in male mice[48].

AXA can stop the inflammatory process through its capacity to reduce the activity of astrocytes, down the progress of reactive oxygen species with decreasing genetic expression of inflammatory enzymes in hippocampus. Also, it conserved the mitochondrial integrity by its ability to regulate the activity of anti-apoptotic Bcl-2 and diminishing the expression of caspase-3[49].

CONCLUSION

Methotrexate has a destructive effect on cerebellar cortex. Vitamin C and astaxanthine improve the damaging effect caused by MTX. Moreover, astaxanthin has a superior defensive effect than vitamin C in prevention of lethal effect of methotrexate on cerebellum of albino rat.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير التحسيني المحتمل لفيتامين ج مقابل الأستازانتين على الضرر الناجم عن الميثوتريكسات فى قشرة المخيخ للفئران: دراسة كيميائية حيوية و نسيجية و كيميائية مناعية

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مقدمة: ان الميثوتريكسات يسبب ضرر في قشرة المخيخ ، ويعتبرفيتامين ج من أحد أكثر مكونات مضادات الأكسدة شيوعًا. قد يكون للأستازانتين دور وقائي ضد الإجهاد التأكسدي في المخ.

الهدف من الدراسة: الغرض من الدراسة هو المقارنة بين الأدوار الوقائية المحتملة لكل من فيتامين ج وألاستازانتين على قشرة المخيخ.

المواد و الطرق: تم الدراسه على ستة وثلاثون من ذكور الجرذان البيضاء و قد قسمت بالتساوي الى المجموعة الضابطة، المجموعة المعالجة بالميثوتريكسات، المجموعه المعالجه ميثوتريكسات + فيتامين ج والمجموعه المعالجه ميثوتريكسات + ألاستازانتين . تم اخذ عينات المخيخ في نهاية اليوم العاشرمن الدراسه. تم تحليل مستوياتMalondialdehyde MDA)) ، الكاتلاز (CAT) ، الجلوتاثيون المنخفض (GSH) وديسموتازالفائق (SOD) في أنسجة المخيخ. تم إجراء الفحص النسيجي المرضي والكيميائي المناعي.

النتائج: الميثوتريكسات يرفع مستوى MDA بشكل ملحوظ ويقلل بشكل كبيرمن مستويات SOD و CAT و GSH في أنسجة المخيخ. الميثوتريكسات يتسبب في تدمير التركيب النسيجي لقشرة المخيخ ويحدث ارتفاعًا ملحوظًا في نشاط مناعة caspase-٣ و GFAP مقارنة بالمجموعةا لضابطه. فيتامين ج وألاستازانتين يخففان بشكل ملحوظ التغيرات البيوكيميائيه التى يسببها الميثوتريكسات .الأستازانتين يقلل بشكل كبيرمن مستوى MDA ويرفع مستويات SOD و CAT و GSH بشكل ملحوظ مقارنة بمجموعة الميثوتريكسات+فيتامين ج. الأستازانتين يحافظ على التركيب النسيجي الطبيعي لقشرة المخيخ ويقلل بشكل كبيرمن النشاط المناعي caspase-٣ و GFAP مقارنة بمجموعة الميثوتريكسات + فيتامين ج.

الخلاصة: لكل من فيتامين ج وألاستازانتين أدوارتحسينية في إصابة قشرة المخيخ التي يسببها الميثوتريكسات و تبين ايضا إن ألاستازانتين أفضل من فيتامين ج في الحماية من تلف قشرة المخيخ.