Original Article

Evaluation of the Role of Growth Hormone Against Cuprizone Induced Multiple Sclerosis in the Cerebellar Cortex of Adult Female Albino Rat (Histological, Immunohistochemical and Radiological Study)

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ABSTRACT

Introduction: Multiple sclerosis (MS) is a primary demyelinating disease which is more common in females in the third and fourth decades of life. The cerebellum is a predilection site for lesion development in MS. Cuprizone is used as an experimental model for toxic demyelination. Growth hormone (GH) is a factor that affect the survival of myelin and the central nervous system cells.

Aim of the Work: The present study aimed to investigate the effect of cuprizone on the cerebellar cortex in an experimental trial to mimic MS and to evaluate the possible protective role of simultaneous administration of recombinant GH.

Material and Methods: Thirty adult female Albino rats were used in the study; weighing from 180- 200 gm and aged from 4 to 6 months. Rats were divided equally into three groups: Group I (Control): rats further subdivided into two subgroups: control A: rats fed with ground standard rodent chow for five weeks and control B: rats followed the same regimen as control A group, in addition to subcutaneous injection daily with 0.1 mL saline for five weeks. Group II (Cuprizone): rats were fed with ground standard rodent chow mixed with 0.2% cuprizone for five weeks. Group III (Cuprizone + GH): rats were fed with ground standard rodent chow mixed with 0.2% cuprizone for five weeks with simultaneous subcutaneous injection with low dose Somatropin (0.4 mg/kg/day).

Results: Cuprizone administration induced histological changes of rats' cerebellar cortex in the form of deeply stained Purkinje cells, apparent increase in perineural spaces with decease in anti-myelin proteolipid protein and synaptophysin immune stain and gliosis by glial fibrillary acidic protein. While simultaneous administration with recombinant GH showed minimal histological changes of the rat's cerebellar cortex.

Conclusion: Simultaneous administration of recombinant GH with cuprizone could help in ameliorating the deleterious effect of cuprizone on rats' cerebellar cortex.

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Key Words: Cerebellum, cuprizone, growth hormone.

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INTRODUCTION

Demyelinating diseases are debilitating diseases that affect patients' quality of life. They include any condition that results in destruction of the myelin sheath of the nerve fibers of the brain, optic nerve, and spinal cord. Multiple sclerosis (MS) is a primary demyelinating disease which is more common in females in the third and fourth decades of life[1].

The cerebellum is a predilection site for lesion development in MS with up to 85% of people with MS experiencing ataxia with or without tremors[2,3].

Symptoms and signs of MS depend upon the site of the lesions. Patients can manifest with a heterogeneous group of symptoms as spasticity, weakness, painful spasms, sensory disturbances, ataxia, optic neuritis, dysphagia, diplopia, cognitive change, and disturbance in balance and coordination. The clinical forms of MS are relapsing remitting MS, secondary progressive MS, primary progressive MS, and progressive relapsing MS[4,5]. Most patients have their symptoms start commonly between the ages of 20 to 40 years[6]. Many medications are used to ameliorate MS symptoms but there is no known cure for MS[5].

Growth hormone (GH) is synthesized and secreted by the anterior lobe of the pituitary gland (somatotroph cells). Growth hormone regulates cell division, regeneration, and proliferation. It affects certain central functions including memory, alertness, motivation, and working capacity. It influences the survival of myelin and the central nervous system cells[7,8].

Cuprizone (a copper chelator) is used as an experimental model for toxic demyelination. Cuprizone model simulates the histopathological data of MS and is a valuable tool in studying demyelination and remyelination[9]. Hence, it became the aim of the present study, to investigate the effect of cuprizone on the cerebellar cortex in an experimental trial to mimic MS and to evaluate the possible protective role of simultaneous administration of recombinant GH.

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MATERIALS AND METHODS

Animals and Experimental design

Thirty adult female Albino rats were included in the study, weighing 180- 200 gm and aged 4-6 months. Rats were purchased from Research Unit Center (Faculty of Medicine, Ain Shams University, Cairo, Egypt). Rats were hosted under routine conditions with free access to food, water, and 12 hours light/ dark cycle. The guidelines of the Ain Shams University Ethics Committee were followed.

Rats were equally divided into three groups

Group I (Control group): included 10 rats that were subdivided into two subgroups: Control A (five rats): rats were fed with ground standard rodent chow for five weeks. Control B (five rats): rats followed the same regimen as control A group, in addition to subcutaneous injection daily with 0.1 mL saline for five weeks.

Group II (Cuprizone group): included 10 rats that were fed with ground standard rodent chow mixed with 0.2% cuprizone for five weeks[10]. Cuprizone (Bis-cyclohexanone Oxaldihydrazone) was purchased in the form of powder (Sigma-Aldrich Inc., St. Louis, MO).

Group III (Cuprizone + GH): included 10 rats that were fed with ground standard rodent chow mixed with 0.2% cuprizone for five weeks. Simultaneous subcutaneous injection of 0.4 mg/kg/day Somatropin (low dose)[11] in the interscapular area was administered. Somatropin is a recombinant human GH (rDNA origin) that is formed of a polypeptide with a sequence of 191 amino-acid residues, identical to that naturally secreted by the pituitary gland. It was purchased in the form of 4 mg vial from SEDICO pharmaceutical co. 6th October city, Cairo, Egypt.

Processing of samples

At the end of the five weeks, rats were subjected to Magnetic Resonance Imaging (MRI, mid axial cut T2 sequence) on the head at Misr Radiology center, Cairo, Egypt. Rats were sacrificed by a lethal dose of ethyl alcohol. The skull was opened, and the cerebellum was meticulously dissected and extracted from the cranial cavity.

The specimens were fixed in 10% buffered formalin and embedded in paraffin blocks. The blocks were sectioned at 5μm with microtome and stained by Hematoxylin and Eosin (Hx. & E.)[12].

For immunohistochemical staining, 5 μm deparaffinized sections were rehydrated and microwaved with citrate buffer (pH 6.0) 10× for 5 minutes then blocked-in phosphate buffered saline for 1h followed by overnight incubation with the primary antibody. The following primary antibodies were used, for myelin proteolipid protein (mouse IgG, Serotec, Düsseldorf, Germany, 1:500), for glial fibrillary acidic protein (GFAP) for astrocytes distribution, monoclonal mouse anti-GFAP (Dako Carpenteria, Ca, USA, 1:500) and for synaptophysin for quantification of synapses, mouse monoclonal mouse antibody (1: 200; Sigma-Aldrich Chemicals). After washing, sections were incubated with biotinylated antimouse IgG, mouse monoclonal antibody (ABC kit, 1:200) and (Sigma-Aldrich Chemicals, 1:200) secondary antibodies respectively for 1 h, Then sections were incubated with avidin-biotin peroxidase complex (ABC Kit, Vector Laboratories). Reactivity was visualized with diamino-3,3 benzidine (Dako Cytomation, Hamburg, Germany). Sections were counterstained using Mayer's hematoxylin (Merck, Damstadt, Germany)[13-15].

Image analysis

Morphometric analysis was performed on H&E-stained slides using Image J software (National Institutes of Health, Bethesda, Maryland, USA). Olympus microscope equipped with a digital camera (BX3M series, Olympus, Tokyo, Japan) was used at Anatomy Department, Faculty of Medicine, Ain Shams University. Ten randomly chosen non overlapping fields in ten sections were obtained from ten different rats of the same group. They were used for measuring area% of GFAP, synaptophysin and anti-myelin proteolipid protein positive immune reaction. Pixels were calibrated for the actual measurements using the stage micrometer. The magnification used was x400 with an objective lens x40. Immune stained areas were masked by a binary color and the area% was then measured.

Statistical analysis

Data analysis was performed using SPSS software (Version 20, IBM Corp., Armonk, NY, USA).) with one-way analysis of variance (ANOVA) followed by the post hoc test with Bonferroni correction to detect the significance between every two groups.

RESULTS

Histological, Immunohistochemical and radiological results

Group I (Control): Light microscopic examination of Hx. & E.-stained sections of cerebellar cortex of the control subgroups IA and IB showed the same regular structure of the three layers of the cerebellar cortex, the outer molecular layer, the middle Purkinje cell layer, and the inner granular layer. The molecular layer was formed mainly of fibers and some glial cells. Purkinje cells appeared as large pyriform cells arranged in one row with vesicular nuclei. The granular layer was formed mainly of small deeply stained granule cells (Figure 1). Immunohistochemically stained sections for anti-myelin proteolipid protein showed strong positive immunoreaction that appeared parallel and homogenously distributed in the molecular layer and scattered in the granular layer (Figure 2). Immunohistochemically stained sections for synaptophysin revealed strong positive immunoreaction in the molecular layer and in the granular layer (Figure 3). While immunohistochemically stained sections for GFAP showed positive immune reaction of scattered small astrocytes with thin processes in the molecular and the granular layers (Figure 4).

MRI mid axial cut T2 sequence magnetic resonance imaging showed the cerebral hemispheres, eyeballs, optic nerves, and the cerebellar hemispheres with homogenous density (Figure 5).

Group II (Cuprizone): Light microscopic examination of Hx. & E.-stained sections of the cerebellar cortex showed deeply stained shrunken Purkinje cells and apparent increase in perineural spaces in the three layers (Figure 6). Immunohistochemically stained sections for anti-myelin proteolipid protein showed weak positive immunoreaction interruptedly distributed in the molecular layer and scattered in the granular layer (Figure 7). Immunohistochemically stained sections for synaptophysin revealed weak positive immunoreaction in the molecular layer and in the granular layer (Figure 8). While immunohistochemically stained sections for GFAP showed strong positive immunoreaction of large astrocytes with thick processes in the molecular and the granular layers (Figure 9).

MRI mid axial cut T2 sequence magnetic resonance imaging showed multiple large hyperintense areas in the cerebellar hemispheres (Figure 10).

Group III (Cuprizone + GH): Light microscopic examination of Hx. & E.-stained sections of cerebellar cortex of group III showed almost organized layers of the cerebellar cortex with apparently minimal perineural spaces around Purkinje cells and granule cells (Figure 11). Immunohistochemically stained sections for anti-myelin proteolipid protein showed strong positive reaction in the molecular layer, which was mainly parallel and homogenously distributed. Small areas of weak interrupted reaction were detected in the same layer. Scattered positive immunoreaction in the granular layer were also noticed (Figure 12). Immunohistochemically stained sections for synaptophysin revealed apparently moderate positive immunoreaction in the molecular layer and in the granular layer (Figure 13). While immunohistochemically stained sections for GFAP showed positive immunoreaction mainly of apparently medium sized astrocytes with thin processes in the molecular and the granular layers (Figure 14).

MRI mid axial cut T2 sequence magnetic resonance imaging showed homogenous density of the cerebellar hemispheres with one or two small hyperintense areas (Figure 15).

Morphometrical results and statistical analysis

Morphometric studies were done for measuring the mean area % of positive immune reaction anti-myelin proteolipid protein, synaptophysin and GFAP of the three groups. Statistical analysis of the measurements revealed highly significant difference between group I and group II for the three measures with a *P-value* <0.001. Similarly, a highly significant difference between group II and group III was revealed for the three measures, with a *P-value* < 0.001. In contrary, non-significant statistical difference between group I and group III for the three measures were revealed with a *P-value* > 0.05 (Table 1). The comparisons between the morphometric results were further illustrated in (Histogram 1).

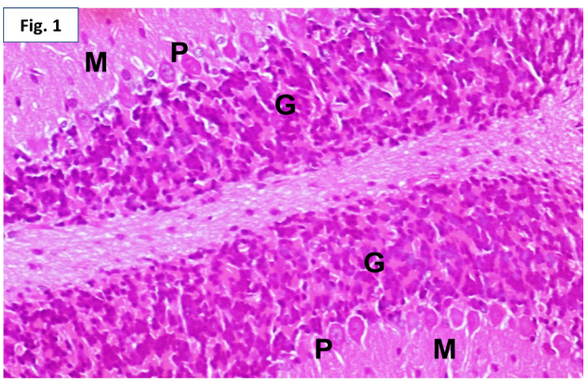


Fig. 1: A photomicrograph of a section of cerebellar cortex of the control group showing the molecular layer (M) formed mainly of fibers and some glial cells, the Purkinje cell layer (P) formed of large pyriform cells arranged in one row with vesicular nuclei and the granular layer (G) formed mainly of deeply stained small granule cells. (Hx. & E., x400)

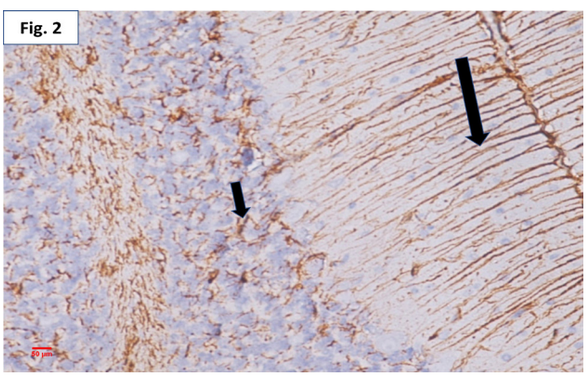


Fig. 2: A photomicrograph of a section of cerebellar cortex of the control group showing strong positive immunoreaction for anti-myelin proteolipid protein that appeared parallel and homogenously distributed in the molecular layer (long black arrow) and scattered between the granule cells in the granular layer (short black arrow). (Anti-myelin proteolipid protein, x400)

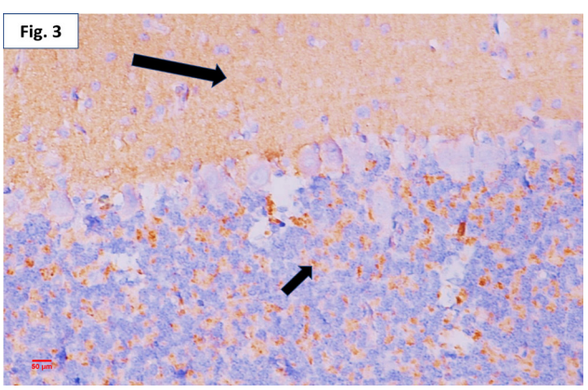


Fig. 3: A photomicrograph of a section of cerebellar cortex of the control group showing strong positive immunoreaction for synaptophysin mainly in the molecular layer (long black arrow) and between the granule cells in the granular layer (short black arrow). (Synaptophysin, x400)

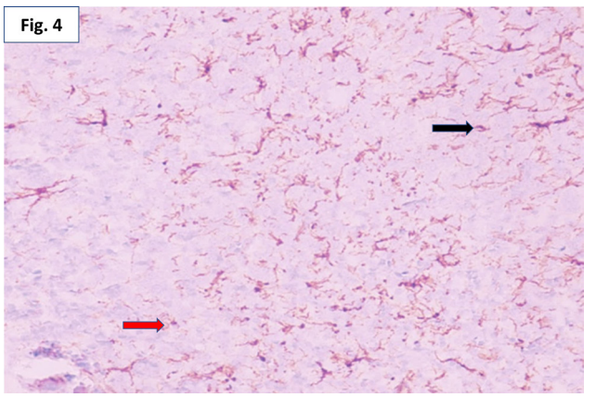


Fig. 4: A photomicrograph of a section of cerebellar cortex of the control group showing positive immunoreaction for GFAP of scattered small astrocytes with thin processes in the molecular layer (long black arrow) and in the granular layer (long red arrow). (GFAP, x400)

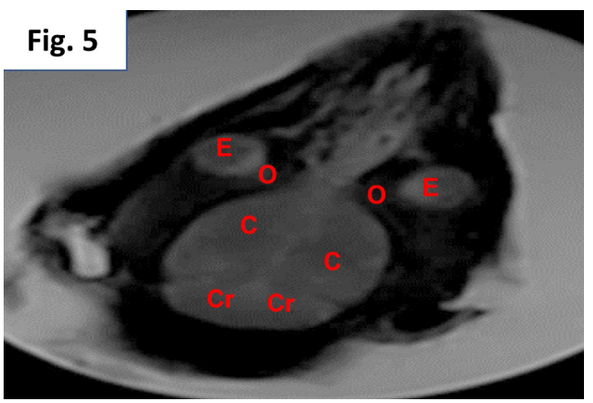


Fig. 5: MRI mid axial cut T2 sequence magnetic resonance imaging of control rat showing two cerebral hemispheres (C), two eyeballs (E), two optic nerves (O) and two cerebellar hemispheres (Cr). Notice the homogenous density of the cerebellar hemispheres. (MRI)

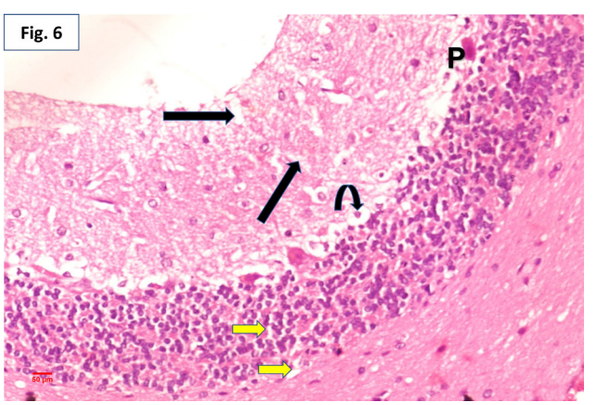


Fig. 6: A photomicrograph of a section of cerebellar cortex of group II showing deeply stained shrunken Purkinje cells (P) and an apparent increase in the perineural spaces around Purkinje cells (curved black arrow), around granule cells (short yellow arrows) and in the molecular layer (long black arrows). (Hx. & E., x400)

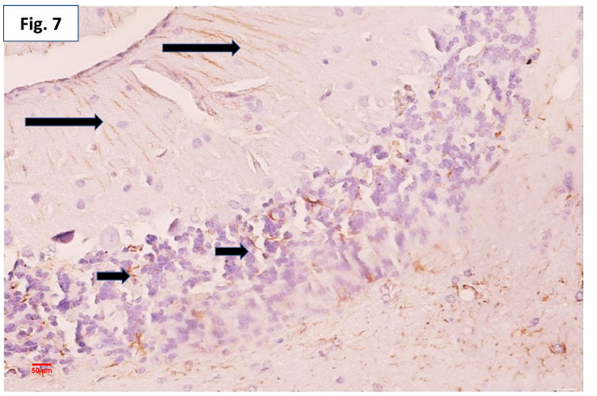


Fig. 7: A photomicrograph of a section of cerebellar cortex of group II showing weak positive immunoreaction for anti-myelin proteolipid protein interruptedly distributed in the molecular layer (long black arrows) and scattered between the granule cells in the granular layer (short black arrows). (Anti-Myelin proteolipid protein, x400)

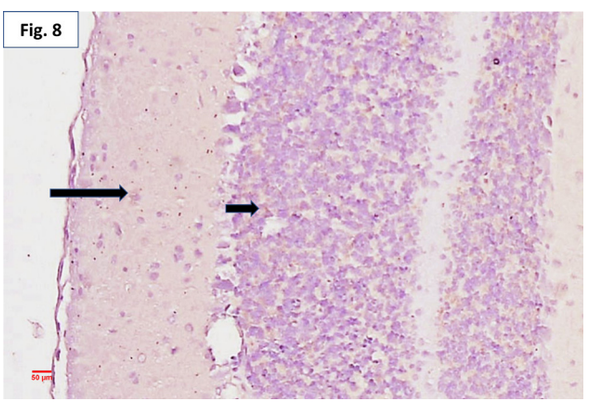


Fig. 8: A photomicrograph of a section of cerebellar cortex of group II showing weak positive immunoreaction for synaptophysin in the molecular layer (long black arrow) and in the granular layer (short black arrow). (Synaptophysin, x400)

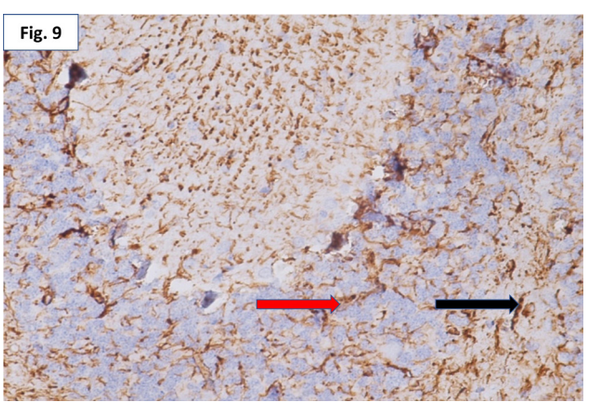


Fig. 9: A photomicrograph of a section of cerebellar cortex of group II showing strong positive immunoreaction for GFAP of large astrocytes with thick processes in the molecular layer (long black arrow) and in the granular layer (long red arrow). (GFAP, x400)

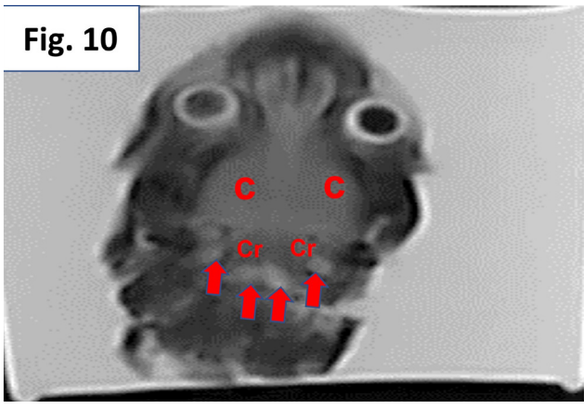


Fig.10: MRI mid axial cut T2 sequence magnetic resonance imaging of group II showing two cerebral hemispheres (C) and two cerebellar hemispheres (Cr) with multiple large hyperintense areas (short red arrows). (MRI)

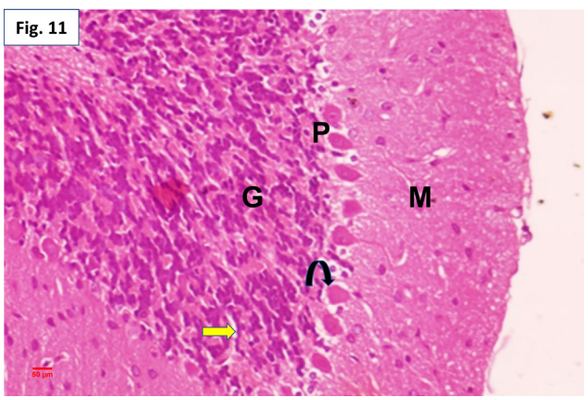


Fig. 11: A photomicrograph of a section of cerebellar cortex of group III showing organized layers of the cerebella cortex. The molecular layer (M), the Purkinje cell layer (P) and the granular layer (G). Notice, apparently minimal perineural spaces around Purkinje cells (curved black arrow) and around granule cells (short yellow arrow). (Hx. & E., x400)

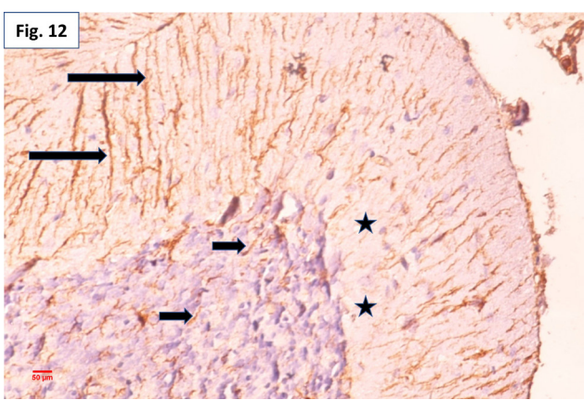


Fig. 12: A photomicrograph of a section of cerebellar cortex of group III showing mainly parallel and homogenously distributed strong positive immunoreaction for anti-myelin proteolipid protein in the molecular layer (long black arrows) with small areas of weak interrupted reaction (black stars) and scattered positive immunoreaction in the granular layer (short black arrows). (Anti-myelin proteolipid protein, x400)

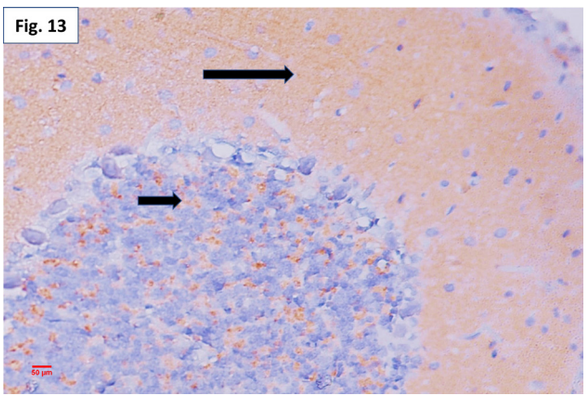


Fig. 13: A photomicrograph of a section of cerebellar cortex of the group III showing apparently moderate positive immunoreaction for synaptophysin in the molecular layer (long black arrow) and in the granular layer (short black arrow). (Synaptophysin, x400)

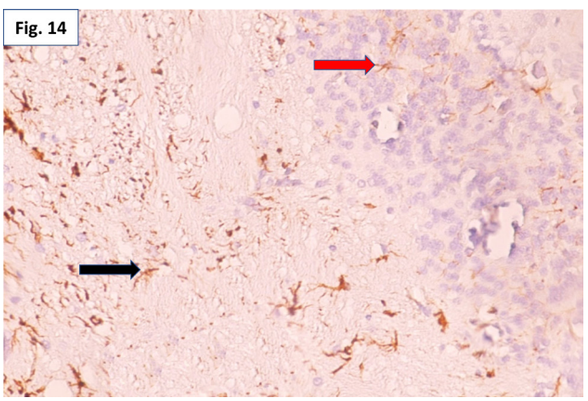


Fig. 14: A photomicrograph of a section of cerebellar cortex of the group III showing positive immunoreaction for GFAP mainly of apparently medium sized astrocytes with thin processes in the molecular layer (long black arrow) and in the granular layer (long red arrow). (GFAP, x400)

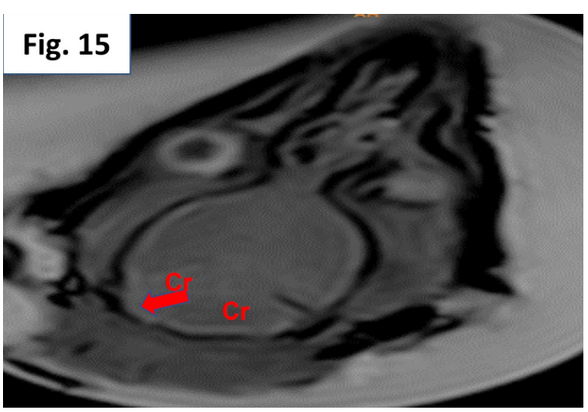
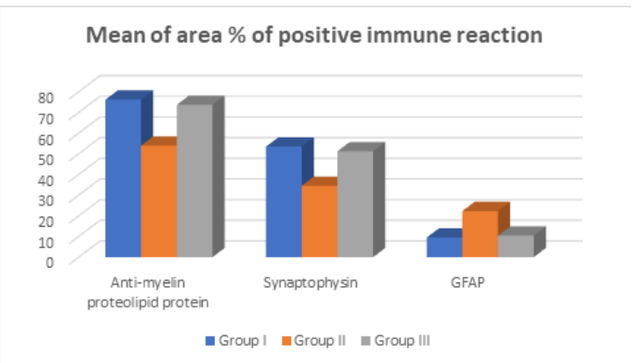


Fig. 15: MRI mid axial cut T2 sequence magnetic resonance imaging of group III showing, homogenous density of the two cerebellar hemispheres (Cr) with one hyperintense small area (short red arrow). (MRI)

Table 1: Comparing the mean area % of anti-myelin proteolipid protein, synaptophysin and GFAP positive immune reaction of the three groups. *P-value*, non-significant (*) and highly significant (**).

	Anti-myelin proteolipid protein positive immune reaction	Synaptophysin positive immune reaction	GFAP positive immune reaction
	Area% (Mean ± Standard deviation)		
Group I (Control)	76.20 ± 1.68	53.48 ± 2.12	9.46 ± 0.35
Group II (Cuprizone)	54.00 ± 3.46	34.56 ± 2.10	22.32 ± 1.43
Group III (Cuprizone +GH)	73.70 ± 1.88	51.24 ± 1.69	10.40 ± 0.98
	(*P-value*)		
Between Group I&II	0.000**	0.000**	0.000**
Between Group II&III	0.000**	0.000**	0.000**
Between Group I&III	0.097*	0.053*	0.152*



Histogram 1: Demonstrating the morphometric comparison between the three groups regarding the mean area% of anti-myelin proteolipid protein, Synaptophysin and GFAP positive immune reaction

DISCUSSION

The cerebellum is a neurological structure that is essential for the smooth purposeful movements. The role of the cerebellum in processing signals for cognition, perception, and emotions has been proved by using the human neuroimaging and animal behavior studies. The cerebellum also has extensive connections with the sub-cortical and the cortical areas which explain the raised possibility that cerebellar dysfunction may lead to motor as well as to non-motor neurological deficits[16]. The cerebellum is considered one of its predilection sites[2] of multiple sclerosis (MS) which is one of the neurodegenerative diseases of the central nervous system that is characterized by demyelination and axonal transection. The prevalence of MS ranges from 5 to 300 per 100 000 people. Multiple sclerosis can lead to a variety of clinical presentation according to the affected areas of the central nervous system. Patients may be presented with numbness or weakness in one or more limbs, cognitive deficits, tremors, unsteady gait, and visual defects[17]. Low blood level of growth hormone (GH) was detected in patients with severe MS[7].

So, the aim of the present study was to investigate the effect of cuprizone on the cerebellar cortex in an experimental trial to mimic MS, and to evaluate the possible ameliorating role of recombinant GH simultaneous administration.

The results of the present study revealed marked histopathological changes of the cerebellar cortex of group II rats that received cuprizone for five weeks as compared to the control group. Light microscopic examination of Hx. & E.-stained sections showed shrunken deeply stained Purkinje cells. An apparent increase in the perineural spaces in the three layers of the cerebellar cortex were also detected. Cuprizone is a copper chelator, and it is used to induce brain lesion and demyelination, as cuprizone alters the cellular respiration by reduction of the cytochrome oxidase activity in the brain[18] also it leads to iron accumulation in the central nervous system with subsequent mitochondrial dysfunction and free radicals' production which contribute to cell death and demyelination[19-21]. Five weeks cuprizone administration in rats is referred to as 'acute' demyelination, denoting a single transient episode of demyelination[22,23].

The previous histological findings of the present study mimic those changes that occur in patients with MS as described by Kemp *et al*.[24] who analyzed post-mortem cerebellar tissues from patients who had multiple sclerosis and reported an increase in Purkinje cell fusion and heterokaryon formation. Giuliani *et al*.[25] also reported loss of Purkinje cell layer with demyelination in multiple sclerosis. In addition, axonal degeneration with MS occurs at disease onset and correlates with the degree of the lesions. This axonal loss may remain clinically silent

for years, and the neurological deficits develop when the compensatory mechanisms of the central nervus system become exhausted[26].

Examination of Hx. & E.-stained sections of group III which received low dose of GH simultaneously with cuprizone showed almost organized layers of the cerebellar cortex with apparently minimal perineural spaces around the Purkinje cells and the granule cells. The protective role of GH on the neurons was explained by Koechling *et al*.[27] who stated that GH increases brain-derived neurotropic factors. Li *et al*.[28] also clarified that exogenous GH showed a protective role for the nervous tissues from neuronal degeneration and the neurocognitive deficits in hypoxic rats.

The immunohistochemical results in the examined sections were confirmed by the morphometric study and the statistical analysis. Examination of immunohistochemically stained sections for anti-myelin proteolipid protein 'The main marker of demyelination of the white matter' of group II revealed weak positive immunoreaction interruptedly distributed in the molecular layer and scattered in the granular layer. Myelin proteolipid protein is the major myelin protein of the central nervous system. It is important in the formation and the maintenance of the multilamellar structure of myelin. The most widely held hypothesis for MS as an autoimmune demyelinating disease is the activation of auto reactive T-cell against myelin-related proteins which affects mainly the white matter of the central nervous system[29]. In contrary, examination of immunohistochemically stained sections for anti-myelin proteolipid protein of group III showed a reaction nearly like that of the control group, strong positive reaction was detected in the molecular layer, which was mainly parallel and homogenously distributed and only small areas of weak interrupted reaction were detected in the same layer. Evidence suggested that neurohormones such as GH are involved in the reparative processes in MS[7]. Growth hormone is known to be a neuroprotective and an antiapoptotic agent, and it has a direct influence on myelination[30].

The demyelinating effect of cuprizone was confirmed by MRI as the cerebellum of group II rats showed multiple large, demyelinated areas. while MRI of the cerebellum of group III rats showed few small, demyelinated areas. High intensity areas on MRI scans of the brain of any mammal reflect lesions that are produced mainly by demyelination and axonal loss[31]. Magnetic resonance imaging has revolutionized the diagnosis of patients with MS, not only by confirming the diagnosis, but also by the follow up that evaluates the response to treatment and determines the disease pattern[32].

Additionally, examination of immunohistochemically stained sections for synaptophysin of group II revealed weak positive immunoreaction in the molecular layer and in the granular layer. While examination of group III revealed moderate positive immunoreaction in the molecular and in the granular layers.

Synaptophysin is a glycoprotein found in the presynaptic vesicle membrane. It is present in all neurons in the brain and spinal cord. The amount of synaptophysin in the presynaptic vesicle membrane reflects the synaptic function[33]. Synaptopathy (Gray matter pathology), represent an early and central feature of MS. Studies supported the hypothesis of synaptic loss role in the pathogenesis of the neurons' disconnection and the loss of their functional reserve that eventually leads to irreversible damage of neurons with MS. Authorized therapies for MS depend on their immune-modulating effect to prevent the development of new inflammatory lesions in both the white matter and the gray matter of the central nervous system[34]. A previous study on rat hippocampus after traumatic brain injury revealed that the treatment with recombinant GH significantly increased the synaptophysin mRNA levels in the hippocampus[35].

Furthermore, examination of immunohistochemically stained sections for GFAP "a marker for astrocytes" of group II clarified astrocytosis in which astrocytes appeared large with thick processes, that agreed with Sen *et al*.[36] who stated that upon cuprizone administration in rats, astrocytes became hypertrophic and activated. The demyelinating process is known to be associated with activation of astrocytes during the active tissue injury state and during the formation of gliotic scars[37]. Astrocytes provide an access of the immune cells to the central nervous system by their ability to function as antigen presenting cells and by their interaction with T cells. In active MS lesions, they acquired a hypertrophic morphology due to massive enlargement of their cellular soma[38]. It was proved that increase astrocyte activity with the increase in their regulatory signaling in the sites of lesions of MS prevent the remyelination at these sites, yet the mechanism is not understood[39]. On the other hand, examination of immunohistochemically stained sections for GFAP of group III showed mainly medium sized astrocytes with thin processes. Wyse and Sernia[40] reported that cultured rat astrocytes derived from hypothalamus and thalamus express angiotensin receptors (AT1a) and GH regulates the function of astrocytes by affecting the transcription of AT1a gene.

CONCLUSION

Simultaneous administration of recombinant GH with cuprizone could help in ameliorating the deleterious effect of cuprizone on rats' cerebellar cortex "a classical rat model of MS" and thus could be beneficial in decreasing the progress of acute MS cases.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تقييم دور هرمون النمو على التصلب المتعدد المستحث بالكوبريزون على قشرة المخيخ فى انثى الجرذ الابيض البالغة (دراسة نسيجية وكيميائية مناعية وإشعاعية)

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مقدمة: التصلب المتعدد هو مرض أولي مزيل للميالين وهو أكثر شيوعًا عند الإناث في العقدين الثالث والرابع من العمر. وقد وجد ان هرمون النمو يؤثرعلى الميالين وخلايا الجهاز العصبي المركزي. يعتبرالمخيخ هو مكان مفضل لتطور مرض التصلب المتعدد ويستخدم الكوبريزون كنموذج اختبارى لإزالة الميالين.

الهدف: هدفت الدراسة الحالية إلى التحقق من تأثير الكوبريزون على قشرة المخيخ فى اناث الجرذان كنموذج تجريبى مشابه للتصلب المتعدد ولتقييم الدور التحسني المحتمل للإعطاء المتزامن لهرمون النمو المؤتلف.

المواد والطرق: تم استخدام ثلاثون أنثى جرذ بالغة في هذه الدراسة. الوزن من ١٨٠ إلى ٢٠٠ جرام وتتراوح الاعمار من ٤ إلى ٦ شهور. تم تقسيم الجرذان بالتساوي إلى ثلاث مجموعات: المجموعة الأولى (المجموعة الضابطة): مقسمة إلى مجموعتين فرعيتين: المجموعة الضابطة أ: تم تغذيتها بطعام القوارض القياسي والمجموعة الضابطة ب: اتبعت الجرذان نفس نظام الغذائى كالمجموعة الضابطة أ مع حقنها تحت الجلد ب٠,١ مل من محلول ملحي لمدة خمسة أسابيع. المجموعة الثانية: تم تغذية الجرذان بطعام القوارض القياسي ممزوج بنسبة ٠,٢٪ كوبريزون لمدة خمسة أسابيع. المجموعة الثالثة: تم تغذية الجرذان بطعام القوارض القياسي المخلوط مع ٠,٢ ٪ كوبريزون لمدة خمسة أسابيع بالتزامن مع حقن جرعة صغيرة من هرمون النمو تحت الجلد (٠,٤ مجم / كجم / يوم).

النتائج: تسبب الكوبريزون في حدوث تغيرات نسيجية في القشرة المخيخية للجرذان وشملت التغيرات التصبغ القاتم لخلايا بيركينجي ، وزيادة واضحة في الفراغات حول الخلايا مع انخفاض فى الصبغة المضاده لبروتين الميالين بينما أظهرت النتائج ان اعطاء هرمون النمو المؤتلف المصاحب لاعطاء الكوبريزون ادى الى القليل فقط من التغييرات النسيجية لقشرة المخيخ فى الجرذان.

الخلاصة: يمكن أن يساعد هرمون النمو المؤتلف في تخفيف التأثير الضار للكوبريزون على قشرة المخيخ في الجرذان.