# The Protective Role of Selenium Against Acrylamide-Induced Cerebellar Neurotoxicity in Adult Male Albino Rats

Original Article

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## ABSTRACT

**Introduction:** Cerebellum is a part of the brain which has about a half of the total number of neurons within the CNS. Acrylamide (ACR) is a chemical compound that is produced naturally in food as a result of high-temperature cooking. It is a potent neurotoxin. Selenium (Se) is an essential element to the good brain function.

**Aim of the Work:** To evaluate the effect of ACR and the possible protective role of Se on the cerebellum of male albino rats.

**Material and Methods:** A total number of 40 male albino rats weighing 250 gm, aging 6 months were used in the study. They were divided into 4 main groups (10 rats each). Group I, the control group, Group II received ACR intraperitoneally. Group III received ACR intraperitoneal and Se orally. Group IV received Se orally. The regimen continued for 4 weeks. The rats were anaesthetized and the cerebellum was extracted, dissected and processed for the light microscopic (LM) and transmission electron microscopic (TEM) examinations as well as morphometric measurements.

**Results:** The LM study of the group II revealed distorted Purkinje cells with pyknotic nuclei and irregularly separated white matter nerve fibers, but in group III, Purkinje cells were similar to the control group with disperse shrunken Purkinje cells. The TEM examination of the group II revealed irregular shrunken Purkinje cells with dark nuclei with distorted cytoplasmic organelles and shrunken granule cells comparing to group III which had normal cytoplasmic organelles of Purkinje and granule cells with few cells with euchromatic nuclei and irregular outline. The immunoreaction for glial fibrillary acidic protein (GFAP) was strong in group II in compared to moderate in group III and weak in group IV.

**Conclusion:** Co-administration of Se with acrylamide is suggested to minimize to a great extent the harmful effects of ACR on the cerebellum of rats.

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**Key Words:** Albino rats, acrylamide, cerebellum, selenium.

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## INTRODUCTION

The cerebellum is one of the main parts of the CNS. About half of the CNS neurons are present in the cerebellum[1]. Acrylamide (ACR) is a chemical compound with the chemical formula C3H5NO. It is a white odourless crystalline solid, soluble in water, ethanol and chloroform[2]. ACR was first synthesized in 1949. It is used for an industrial purpose as gel electrophoresis and papermaking[3]. ACR is a substance that is produced naturally in starchy foods as a result of high-temperature cooking[4]. It has been found in a wide variety of cooked foods, such as bread and potatoes, crisps, biscuits and coffee[5]. Moreover, at low levels, ACR is a potent neurotoxin affecting both central and peripheral nervous systems[6]. The higher affinity of ACR to form adducts with glutathione, proteins, and DNA directly or after metabolized to its epoxide, glycidamide (2, 3-epoxy1propanamide) attributes to its neurotoxicity[7]. In addition, the ability of ACR to form haemoglobin adduct led to dysfunction of oxygen transport causing hypoxia led to vascular disturbance[8]. ACR exposure linked to nerve terminal damage, reduction of the antioxidative capacity and oxidative stress in rat cerebellum[9].

Selenium (Se) is a trace element that is essential to good health, but required only in a small amount[10]. The plant foods, animal kidneys, seafood, egg yolk and Brazil nuts are considered the major dietary sources of Se[11]. Se has an essential role in the brain due to its direct antioxidant role and via its participation in maintaining redox balance, mitochondrial dynamics, regulation of Ca2+ channels and modulation of neurogenesis[12]. Se is an essential cofactor to numerous antioxidant enzymes especially, glutathione peroxidase (GSH-Px) which prevents cellular damage from free radicals[13].

## AIM OF THE WORK

The present work was done to study the effect of ACR administration on the histological structure of the rat cerebellum and to assess the possible protective role of Se on rat cerebellar injury under the influence of acrylamide.

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## MATERIAL AND METHODS

### *Ethical approval*

The approval of the study protocol was done by the Ethical Committee of the Faculty of Medicine, Helwan University, Egypt (Serial number: 23 -2021).

### *Drugs*

ACR powder was purchased from Sigma–Aldrich Co. (99% pure). Se powder was purchased from Sigma–Aldrich Co. (98.5% pure). Rabbit anti-cow polyclonal antibody against GFAP (Cat. No. IR524, Dako; Carpenteria, CA).

### *Animals*

Forty adult male albino rats aging 6 months were used in this study. Each rat was weighing 200 – 250 gm. The rats were bred in Pharmacology Department at Helwan University, Cairo, Egypt. They were treated according to the Guidelines of Helwan Committee of Animal Research Ethics (CARE).

### *Experimental design*

The rats were settled in well-ventilated stainless steel cages for 4 weeks. They were separated into 4 groups (each consisted of 10 rats). Group I "control" consists of 2 subgroups; Group Ia; negative control and group Ib; positive control: 5 rats received saline intraperitoneally for 4 weeks. Group II received ACR (30 mg/kg body weight/ day for 5 successive days each week) for 4 weeks. Group III received ACR (30 mg/kg bw./day for 5 successive days each week) intraperitoneally and Se (0.1 mg/kg bw. daily) orally for 4 weeks. Group IV received Se (0.1 mg/kg bw. daily) for 4 weeks orally. The used dose in this work was according to previous studies[14,15].

At the end of the experimental period, the rats were anesthetized by inhalation using diethyl ether then sacrificed. The head was incised and specimens of the cerebellum were removed and prepared for examination.

### *Light microscopic (LM) study*

The collected specimens were fixed in 10% neutral formalin over one night. After fixation, the tissues were dehydrated, cleared then embedded in paraffin blocks. Serial sections of 5µm thickness were cut and stained with hematoxylin and eosin (H&E) for routine histological examination[16].

### *Glial fibrillary Acidic Protein (GFAP) Ab-1 (clone GA-5)*

Paraffin-embedded sections were washed in a series of xylene and alcohol baths, then rinsed in 0.1 M PBS and blocked by 10% normal goat serum then incubated with a rabbit anti-cow polyclonal antibody against GFAP for 48 h at 4°C. The sections then were sequentially incubated with biotinylated goat anti-rabbit IgG for 90 min, and then with 0.2% ABC-Elite reagent for 90 min at room temperature. Reaction products were visualized with DAB[17].

### *Transmission electron microscopic (TEM) study*

The samples were fixed in 3% glutaraldehyde for 2 h at room temperature, rinsed, then post-fixed in 1% osmium tetroxide for 2 h at room temperature. The samples were dehydrated in an ethanol series and finally with an absolute ethanol for 30 min. The samples were infiltrated with epoxy resin and acetone. Ultrathin sections were collected and the sections were then double stained in uranyl acetate followed by lead citrate[18].

The stained sections were examined with a JEOL JEM 1010 transmission electron microscope at 70 kV at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

### *Morphometric study*

The data was measured on H&E-stained sections in ten non overlapping randomly chosen fields. Data were obtained using "Leica Qwin 500 C" image analyzer computer system Ltd. (Cambridge, England) in Histology department, Faculty of Medicine, Ain shams University.

1. Number of Purkinje cells.
2. Thickness of granular cell layer.
3. Area percentage of GFAP-positive immunoreactive astrocytes in different layers of the cerebellum.

### *Statistical analysis*

The above parameters were calculated for animals in each group studied. The mean value and standard deviation were calculated for each parameter. The SPSS statistical program version 20 (IBM corporation, New York, USA) was used. One way ANOVA was employed to compare the means in the different groups with each other, followed by Post Hoc test to detect the significance between every 2 groups where a *P-value* < 0.05 is considered significant[19].

## RESULTS

### *The morphometric study*

#### The count of Purkinje cells

The mean count of Purkinje cells of group II (Power 40) showed a significant decrease when compared with that of group I. The mean count of Purkinje cells of group III showed a significant increase when compared with that of group II and a significant decrease when compared with that of group I. The mean count of Purkinje cells of group IV showed a non-significant increase when compared with that of control group (Table 1; Histogram 1).

#### The thickness of the granular layer (µm)

The mean thickness of the granular layer of group II showed a significant decrease when compared with that of group I. The mean thickness of the granular layer of group III showed a significant increase when compared with that of group II and a significant decrease when compared with that of group I. The mean thickness of the granular layer of group IV showed a non-significant increase

when compared with that of the control group (Table 2; Histogram 2).

**The area percentage (%) of GFAP-positive immunoreactive astrocytes in different layers of the cerebellum**

The mean area percentage of GFAP of group II showed a significant increase when compared with that of group I. The mean area percentage of GFAP of group III showed a significant decrease when compared with that of group II and a significant increase when compared with that of group I. The mean area percentage of GFAP of group IV showed a non-significant increase when compared with that of the control group (Table 3, Histogram 3).

## *Light microscopic study*

**H&E-stained sections**

The examination of the cerebellar cortex of group I (control group) showed outer molecular (M), middle Purkinje cell (P), and inner granular (G) layers. Purkinje cells were arranged in one row. The granule cells were appeared numerous with dark rounded nuclei. The white matter had closely packed nerve fibers (Figures AI, AII). On the other hand, group II examination showed distorted Purkinje cells with pyknotic nuclei. The molecular layer had pericellular halos. The nerve fibers were irregularly arranged in the white matter (Figures BI, BII). Group III showed some Purkinje cells with normal shape, but others appeared shrunken with pyknotic nuclei. The white matter had less separated nerve fibers (Figures CI, CII). Group IV showed normal shape of Purkinje cells and granular cells similar to the control group. The white matter had closely packed nerve fibers similar to the control group (Figure D).

**Immunohistochemical study for GFAP**

A section in a rat cerebellar cortex of the Control Group showed a weak positive GFAP immunoreaction in the cytoplasm and the processes of astrocytes (Figure E). They were few in the granular, molecular and Purkinje cell layers; while it was strong in group II and moderate in group III. The immunoreactivity in the group IV was weak similar to the control group (Figures F, G, H).

**Electron microscopic study**

Examination of the ultrathin sections of the cerebellar cortex of the control rats revealed that Purkinje cell with an euchromatic nucleus (N). The cytoplasm showed multiple mitochondria (M) with regular cristae. The myelinated nerve fibers in the granular layer had a regular compact myelin sheath (Figures II, I II). An examination of the ultrathin sections of group II revealed a shrunken Purkinje cell with an irregular ill-defined nucleus (N). The cytoplasm contained dilated RER cisternae and many mitochondria. The granule cells were shrunken with dense heterochromatin and ill-defined cell membrane. Some myelinated nerve fibers of the granular layer showed disruption of myelin sheath (Figures JI, JII). The examination of group III revealed a Purkinje cell was apparently within average size with an euchromatic nucleus and irregular outline. The granule cell nuclei were similar to the control group. Some myelinated nerve fibers had regular compact myelin sheath, but others had splitting myelin sheath (Figures KI, KII). On the other hand, examination of ultrathin sections of the cerebellar cortex of rats in group IV revealed Purkinje cells with euchromatic nuclei similar to the control group. The granule cells had nuclei containing clumps of heterochromatin (Figure L).

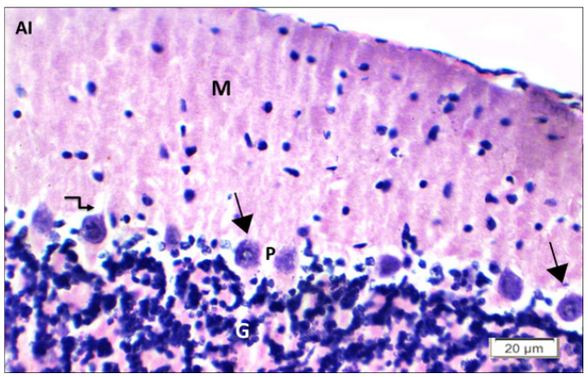



**Fig. A1:** A photograph of a section in the cerebellar cortex of an adult albino rat of the control group showing outer molecular (M), middle Purkinje cell (arrows), and inner granular (G) layers. The Purkinje cells (arrows) are arranged in one row. They are pyriform shaped with a basophilic granular cytoplasm, central vesicular nuclei and prominent nucleoli. (H&E x400)

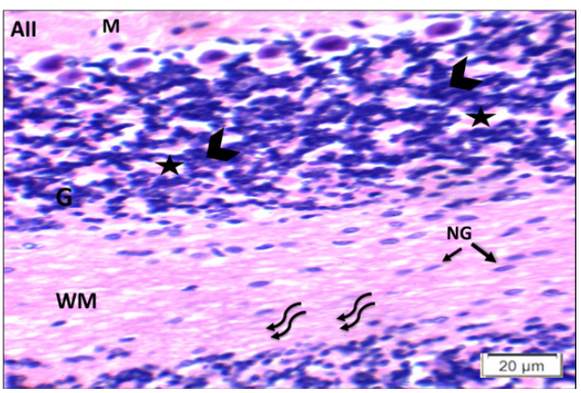



**Fig. A11:** A photograph of section in the cerebellar cortex of an adult albino rat of the control group showing that the granule cells (G) (arrow heads) appear numerous, closely packed with dark rounded nuclei and acidophilic cerebellar islands (asterisks) in-between. The white matter (WM) consisting of bundles of closely packed nerve fibers (double wavy arrows) run in different directions and neuroglial cells (NG) in between. (H&E x400)

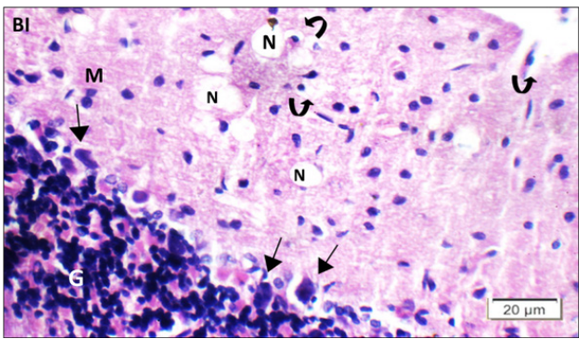



**Fig. B1:** A photograph of a section in the cerebellar cortex of an adult albino rat of Group II showing that the Purkinje cells are shrunken, distorted and deeply stained (arrows). Notice pericellular halos (curved arrow) in the molecular layer (M) and vacuolations of the neuropil (N) of the molecular layer. (H&E x 400)

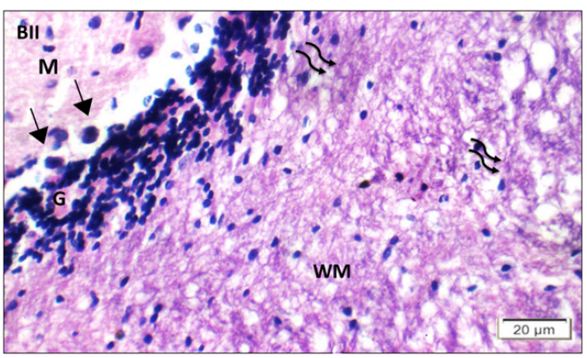



**Fig. B11:** A photograph of a section in the cerebellar cortex of an adult albino rat of Group II showing that the white matter (WM) has widely separated and irregularly arranged nerve fibers (double wavy arrows). Notice irregular shaped deeply stained Purkinje cells (arrows) surrounded by vacuolated neuropil (stars). (H&E x400)

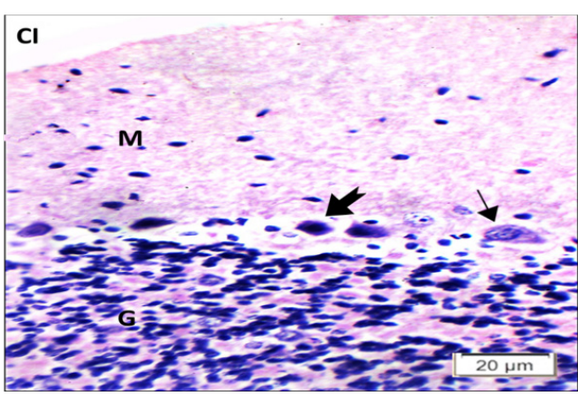



**Fig. C1:** A photograph of a section in the cerebellar cortex of an adult albino rat of Group III showing that some Purkinje cells appear with normal shapes, basophilic granular cytoplasm, vesicular nuclei and prominent nucleoli (arrows). Others appear shrunken with a deeply stained cytoplasm and an irregular in shape with pyknotic nuclei (bifid arrow). Notice a minimal vacuolation of neuropil (stars) in the Purkinje cell layer. The molecular layer is similar to that of the control group (H&E x400)

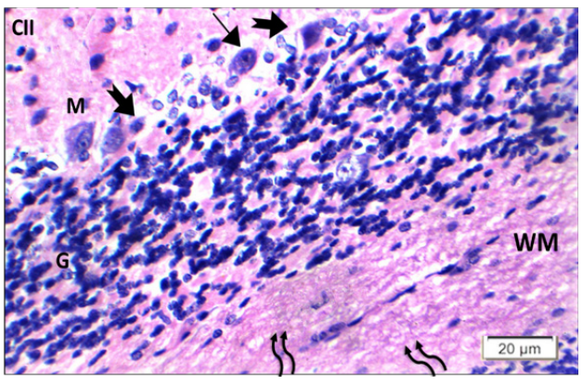



**Fig. C11:** The white matter (WM) has less separated fibers (double wavy arrows) than group II (acrylamide administrated group). Notice some Purkinje cells (arrows) appear normal with a basophilic granular cytoplasm and vesicular nuclei. Others (bifid arrows) are deeply stained, shrunken and irregular in shape with pyknotic nuclei. (H&E x400)

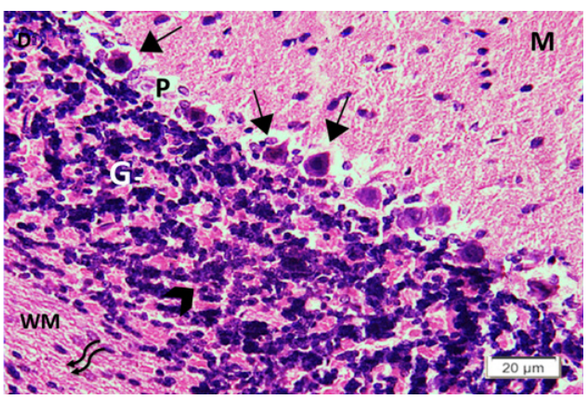



**Fig. D:** A photograph in the cerebellar cortex of an adult albino rat of Group IV showing that the Purkinje cells (arrows) arranged in one row. They are pyriform shaped with a basophilic granular cytoplasm, central vesicular nuclei and prominent nucleoli are similar to the control group. The granular layer (G) are composed of numerous closely packed granule cells (arrow head). The molecular layer (M) showed superficial stellate cells and deep basket cells separated by lightly acidophilic neuropil. In white matter (WM) the nerve fibers are closely packed (double arrows). Notice the arrangement of cells of the granular layer (G) and the molecular layer (M) are similar to the control group. (H&E x400)

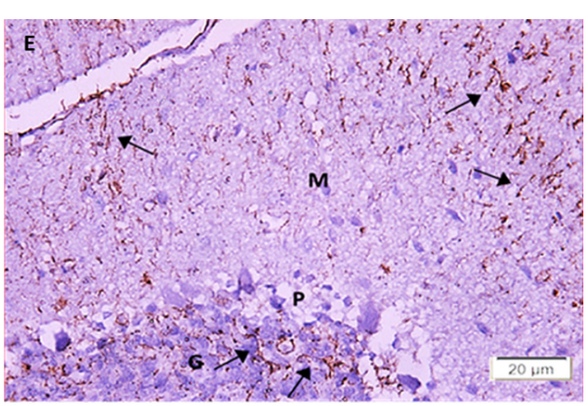



**Fig. E:** A photograph of a section in the cerebellar cortex of an adult albino rat of the control group showing a weak positive GFAP immunoreaction in the cytoplasm and the processes of astrocytes (arrows). They are few in the granular (G), molecular (M) and Purkinje cell (P) layers. (GFAP Immunostain x400)

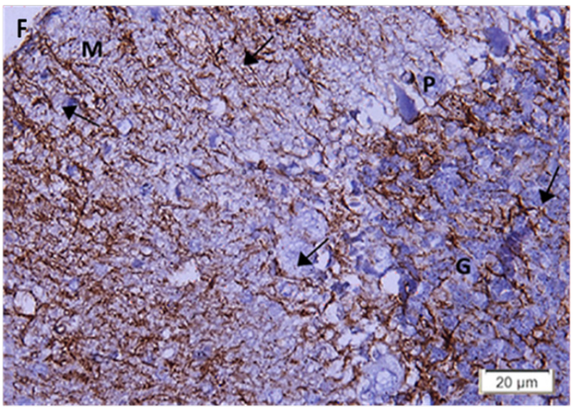



**Fig. F:** A photograph of a section in the cerebellar cortex of an adult albino rat of group II showing a strong positive GFAP immunoreaction in cytoplasm and processes of astrocytes (arrows) in the granular (G), molecular (M) and Purkinje cell (P) layers. (GFAP Immunostain x400)

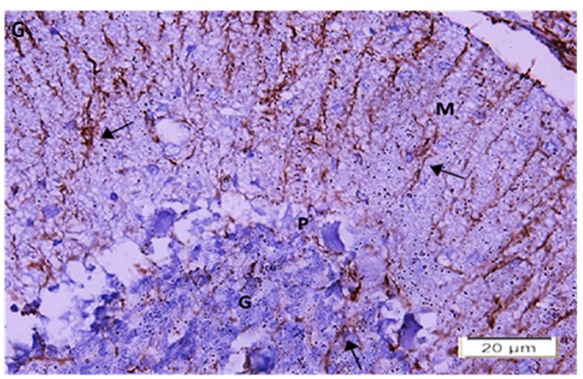



**Fig. G:** A photograph of a section in the cerebellar cortex of an adult albino rat of group III showing a moderate positive GFAP immunoreaction in the cytoplasm and the processes of astrocytes (arrows) that appear numerous in the granular (G), molecular (M) and Purkinje cell (P) layers. (GFAP Immunostain x400)

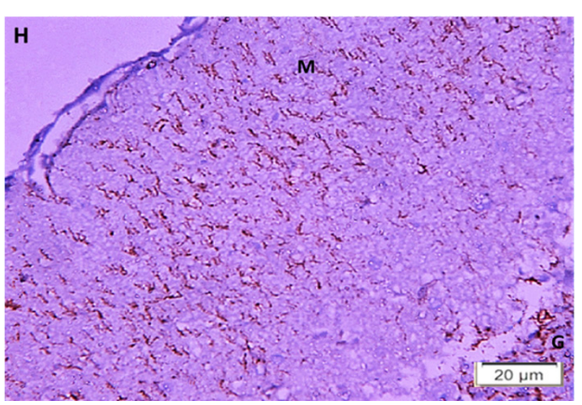



**Fig. H:** A photograph of a section in the cerebellar cortex of an adult albino rat of group IV showing a weak positive GFAP immunoreaction in the cytoplasm and the processes of astrocytes (arrows). They are few in the granular (G), molecular (M) and Purkinje cell (P) layers. (GFAP Immunostain x400)

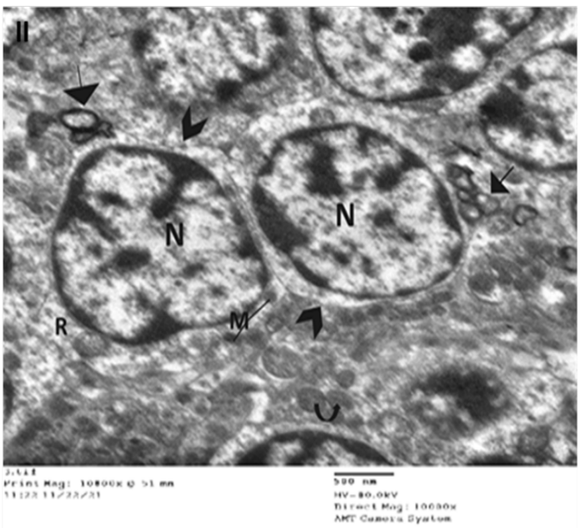



**Fig. i I:** A photomicrograph of an ultrathin section in the cerebellar cortex of an adult albino rat of the control group showing that the myelinated nerve fibers have a regular compact myelin sheath (arrow with thick head) and multiple mitochondria (M) within the axoplasm. Notice parts of two granule cell nuclei (N) surrounded by an intact cell membrane (arrow head) with a thin rim of cytoplasm containing mitochondria (M) and free ribosomes (R). Notice also mitochondria (curved arrows) in the neuropil between the granule cells. (TEM x10000)

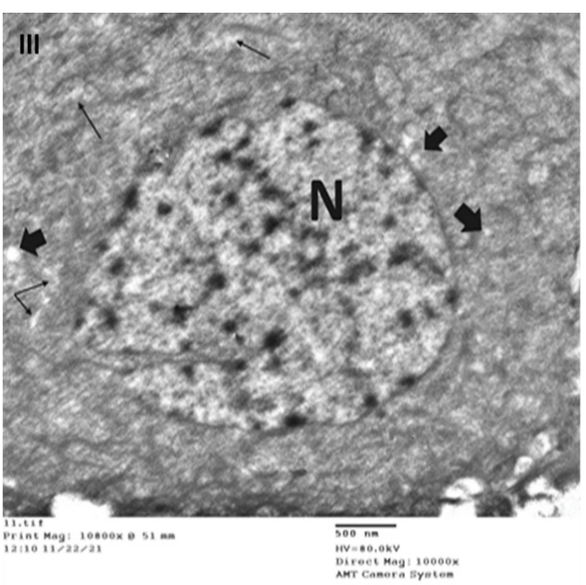



**Fig. i II :** A photomicrograph of an ultrathin section in the cerebellar cortex of an adult albino rat of the control group showing that the Purkinje cell has euchromatic nucleus (N), cisternae of rough endoplasmic reticulum (arrows) and Golgi apparatus (thick arrows). (TEM x10000)

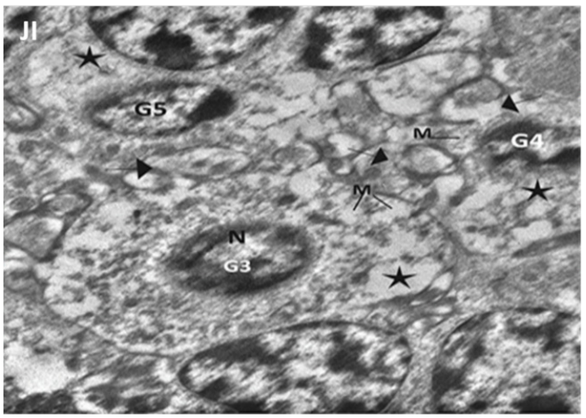



**Fig. JI:** A photomicrograph of an ultrathin section in the granule cell layer of the cerebellar cortex of an adult albino rat of group II showing the granule cell layer showing myelinated nerve fibers with a disrupted myelin sheath (arrow with thick head). Some granule cells show shrunken nuclei as in granule cells G3, G4 and G5. Some granule cells exhibit rarified cytoplasm (asterisks). Notice mitochondria with destroyed cristae (M). (TEM x10000)

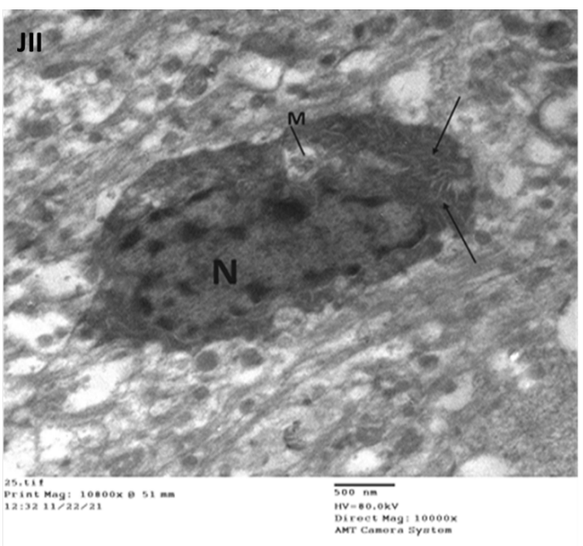



**Fig. JII:** A photomicrograph of an ultrathin section in the cerebellar cortex of an adult albino rat of group II showing the marked shrunken Purkinje cell with an irregular ill-defined dark nucleus (N). The cytoplasm contains a shrunken RER with dilated cisternae (arrows) and mitochondria (M) with destroyed cristae. (TEM x10000)

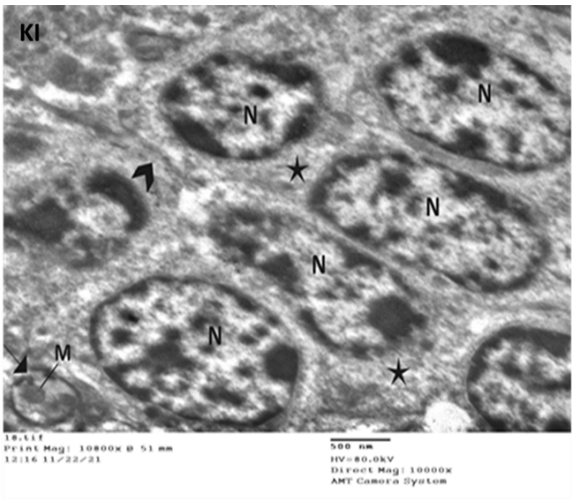



**Fig. KI:** A photomicrograph of an ultrathin section in the cerebellar cortex of an adult albino rat of group III showing some granule cells that have an intact cell membrane (arrow head). Some myelinated nerve fibers have regular compact myelin sheath (arrow with thick head) with mitochondria (M) within axoplasm. Notice areas of rarefied cytoplasm (asterisks) are seen. (TEM x10000)

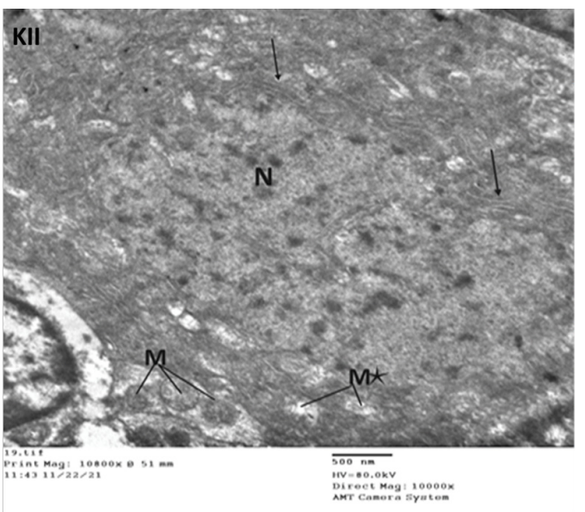



**Fig. KII:** A photomicrograph of an ultrathin section in the cerebellar cortex of an adult albino rat of group III showing the Purkinje cell that has an euchromatic nucleus (N) with an irregular outline. Cisternae of rough endoplasmic reticulum appears similar to the control group (arrow). The cytoplasm shows most mitochondria with regular cristae (M) while others with destroyed cristae (M*). (TEM x10000)

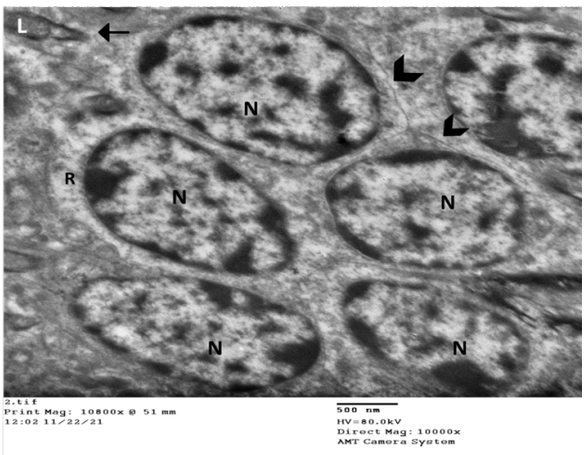



**Fig. L:** A photomicrograph of an ultrathin section in the cerebellar cortex of an adult albino rat of group IV showing the myelinated nerve fibers in the granular layer has a regular compact myelin sheath (arrow with thick head). Notice granule cell nuclei (N) surrounded by an intact cell membrane (arrow head) with a thin rim of cytoplasm containing free ribosomes (R). (TEM x10000)

**Table 1:** The mean ± SD of the count of Purkinje cells (Power 40) of the control and experimental groups

| Groups | Mean Count of Purkinje Cells ± SD | *P value* |
|---|---|---|
| Group I | 9.70 ± 1.34 | |
| Group II | 3.00 ± 1.05 | 0.000** |
| Group III | 5.60 ± 0.70 | 0.000** |
| Group IV | 10.40 ± 1.26 | 0.170 |

*P-value* > 0.05: Non significant **P-value* < 0.05: Significant
***P-value* < 0.01: Highly significant All are compared to group I (control group) SD: standard deviation

**Table 2:** The mean ± SD of the thickness of granular layer (µm) of control and experimental groups

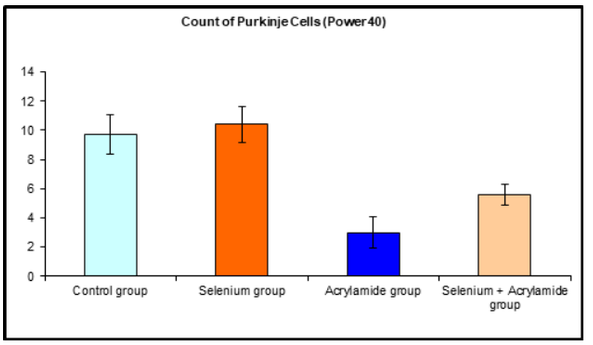
| Groups | Mean of thickness of granular layer ± SD (µm) | *P value* |
|---|---|---|
| Group I | 137.77±11.58 | |
| Group II | 72.75±8.94 | 0.000** |
| Group III | 101.20±9.28 | 0.000** |
| Group IV | 134.06±16.85 | 0.497 |

*P-value* > 0.05: Non significant **P-value* < 0.05: Significant
***P-value* < 0.01: Highly significant All are compared to group I (control group)

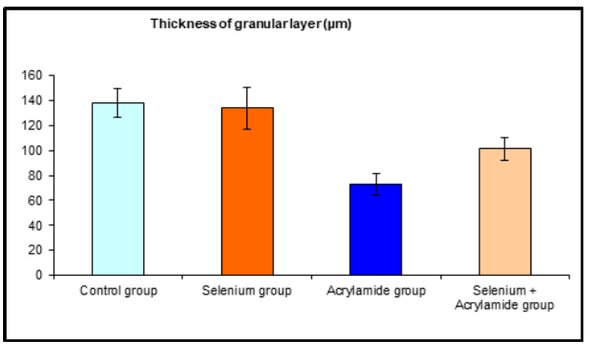
**Table 3:** The mean ± SD of the area percentage of GFAP of the control and experimental groups

| Groups | Mean of area percentage of GFAP ± SD (%) | *P value* |
|---|---|---|
| Group I | 3.93 ± 1.17 | |
| Group II | 19.33 ± 3.81 | 0.000** |
| Group III | 9.38 ± 2.17 | 0.000** |
| Group IV | 4.29 ± 1.61 | 0.743 |

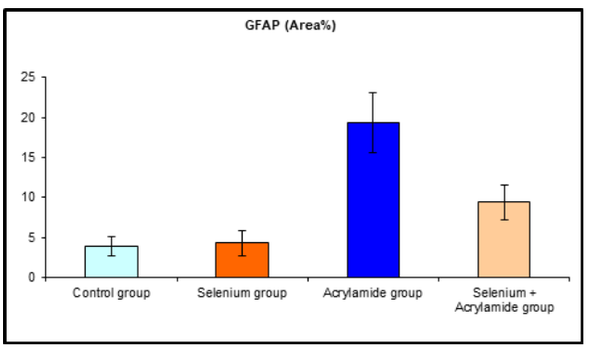
*P-value* > 0.05: Non significant **P-value* < 0.05: Significant
***P-value* < 0.01: Highly significant All are compared to group I (control group)




**Histogram 1:** The mean ± SD of the count of Purkinje cells of the control and the studied groups




**Histogram 2:** The mean ± SD of the thickness of granular layer (µm) of the control and the studied groups.




**Histogram 3:** The mean ± SD of the area percentage of GFAP (%) of the control and the studied groups.

## DISCUSSION

The Purkinje cells are the sole efferent source of the cerebellum and their output is responsible for coordination of somatic motor activity and for regulation of muscle tone and equilibrium[20]. Thus, it is anticipated that toxic compromise of Purkinje function would significantly impact gait leading to an abnormal gait and muscle weakness[21]. ACR is considered one of the neurotoxins that causes Purkinje cells death[22]. Male animals were used in this study to avoid any potential influence of hormones produced during the estrous cycle on the results[23].

In the present study, light microscopic examination of ACR treated rats revealed marked histopathological changes specifically of Purkinje cells. In ACR administrated group, the Purkinje cells were distorted with pyknotic nuclei. Some of them disappeared leaving empty spaces. The degenerated cells could be explained by ACR-induced Purkinje cell injury through its direct effect on intracellular $Ca^{2+}$ regulation or membrane targeting processes[4,24,25]. Empty vacuolated spaces could be explained by the ability of ACR to induce oxidative stress, ROS generation, mitochondrial dysfunction and cytoskeleton changes resulted in cell death necrosis or apoptosis[26,27]. Wherein, ROS interacts with polyunsaturated fatty acids of the cell membranes promoting their disintegration and loss of their permeability resulting in a loss of the pyriform shape of some Purkinje cells and disappearance of others vacuolization[28,29]. The examination of ACR treated group also revealed separation among the cells of molecular layer, pericellular halos and vacuolations of their neuropil of molecular layer. Similar findings were observed by Rawi *et al*. and Amin *et al*.[30,31]. The thickness of granular layer was decreased statistically, this finding was approved by Abdel Mohsen *et al*. (2020)[32] who studied the damaging effects of cisplatin on the cerebellum by neuronal loss. In this study, the cerebellar white matter fibers were irregularly arranged and separated. This was explained by Elblehi *et al*. (2020)[33] who stated that ACR could cross easily neuronal membrane, which provokes ROS production mediated mitochondrial damage and disruption of mitochondrial permeability resulting in the release of key apoptotic proteins, including cytochrome-c and caspase-3 causing demyelination, axonal shrinkage, neurofibrillary accumulations, and vacuolar changes in the white matter. In EM examination of ACR treated group the cytoplasm of the most of Purkinje cells contained dilated RER cisternae and swollen mitochondria with destroyed cristae. Some granule cells showed pyknotic nuclei. The myelinated nerve fibers of the granular layer revealed disruption of the myelin sheath. These findings are in accordance with the results of Zhang *et al*., 2017 and Wang *et al*., 2021[34,35]. Endoplasmic reticulum stress (ERS) is a cytoprotector of cells against defects. In case of disruption of cellular homeostasis, ERS acts as an apoptotic executor to initiate the neuron apoptosis[4].

It was discovered that ACR causes cerebellar injury through inducing microtubule abnormalities, neuronal apoptosis and neurotrophic deficiency. Microtubule-associated protein (tau) is enriched in neuronal axons which maintain the normal structure and function of the neurons by mediating axonal transport, synaptic structure and neurotransmission[36,37]. Tau can be post-translationally modified by phosphorylation. The phosphorylation state is regulated by various kinases, among which glycogen synthase kinase 3β (GSK3β) kinase has a prominent role[38]. ACR exposure decreases the activity of (GSK3β) which leads to an excessive phosphorylation of tau[4]. This accelerates the microtubule depolymerization, interferes with the axonal transport, increases the self-assembly propensity to form toxic oligomeric species and finally leads to the neuronal degeneration[39].

Se has an antioxidant effect due to its efficacy in maintaining the redox homeostasis by being cofactor to glutathione peroxidase (GSH-Px) enzyme and reducing the lipid peroxidation[13]. The antioxidant property of Se could be explained by its ability to regenerate the membrane-bound enzymes and inhibit apoptosis[40]. This explains the histological improvement of ACR & Se administrated group in comparison to the ACR administrated group. The changes were in the number and viability of Purkinje cells, granular layer and molecular layer appeared similar to the control group and the fibers of white matter became less separated. This improvement was also approved by Elwej *et al*., (2018)[41] who studied the effect of Se on the cerebellar injury. EMT cerebellar examination of ACR and Se treated rats revealed that most of the Purkinje cells were apparently normal with euchromatic nuclei. Some Purkinje cells showed euchromatic nuclei with irregular outline and mitochondria with destroyed cristae. The granule cells appeared with nuclei containing clumps of heterochromatin and surrounded by a thin rim of cytoplasm. Most of the myelinated nerve fibers had a regular compact the myelin sheath and normal mitochondria. This improvement was approved by Soudani *et al*., (2012)[42] and Hegazy *et al*., (2023)[43] who studied the protective effect of Se on neurotoxicity caused by lead on the rat cerebellum and by lead on the rat cerebrum, respectively. ACR causes a significant reduction in monoamine neurotransmitters as norepinephrine (NE), epinephrine (EPI) and serotonin (SER) in the brain tissue through increasing the level of ROS which increases the activity of MAO enzyme (Monoamine oxidase enzyme) and through the axonal and nerve terminal degeneration which causes alternations in the synthesis of transmitter, storage uptake, release and reduction in the synaptic vesicles. There was an increase in the levels of monoamine neurotransmitters in brain tissue after Se administration. This protective effect may be due to the antioxidant property that decreases the brain oxidative stress and the lipid peroxidation hence the brain MAO activity was decreased leading to an increase in the level of monoamine brain neurotransmitters (EPI, NE, SER)[44,45]. Abou Zaid *et al*., (2017)[46] approved that ACR affects brain cognitive function by progressively enhancing the activity of acetylcholine esterase enzyme hence faster acetylcholine degradation and consequently lowered stimulation of acetylcholine receptors, which cause a reduction of the diverse cholinergic and non-cholinergic function. This effect was improved significantly after Se administration.

In this study, the immunohistochemical examination revealed a strong positive GFAP in the ACR administrated rats. There was an increased area% of GFAP positive cells in the cerebellum. The ACR-induced excessive lipid peroxidation, elevation of ROS and reduction in antioxidants which increase the incidence of inflammatory reactions in the neural tissues leading to a strong reactive

astrogliosis[47,48]. On the other side, the immunoreaction for GFAP in group III was a weak to a moderate in the cytoplasm and the processes of few astrocytes in all cerebellar layers. The mean area percentage of these cells showed a statistically significant decrease as compared with that of group II and a significant increase as compared with the control group. The immunoreaction for GFAP was weak positive in the cytoplasm and the processes of astrocytes in all cerebellar layers. This was confirmed statistically by a non-significant increase in the mean area percent of these cells as compared with the control group.

## CONCLUSION

In conclusion, ACR had a neurotoxic effect on all layers of the grey matter (molecular, Purkinje cell and granular layers) as well as the white matter of the cerebellum. Co-administration of Se with acrylamide minimize to a great extent the harmful effects of ACR on the histological structure of cerebellum. However, further experimental studies on Se with regard to different doses, frequency and duration of administration are recommended to protect human nerve tissue from ACR neurotoxicity.

## CONFILCT OF INTERESTS

There are no conflicts of interest.

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**الملخص العربى**

# الدور الوقائي للسيلينيوم ضد السمية العصبية الناتجة عن مادة الأكريلاميد في ذكور الجرذان البيضاء البالغين

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**المقدمة:** المخيخ هو جزء من الدماغ، يحتوي على حوالي نصف العدد الإجمالي للخلايا العصبية داخل الجهاز العصبي المركزي. مادة الأكريلاميد هي مركب كيميائي ينتج بشكل طبيعي في الأطعمة نتيجة الطهي على درجة حرارة عالية. وهو سم عصبي قوي. السيلينيوم هو عنصر أساسي ضروري لأداء المخ لوظائفه جيدا.

**الهدف من البحث:** تقييم تأثير مادة الأكريلاميد، و الدور الوقائي المحتمل للسيلينيوم على المخيخ في الجرذان البيضاء البالغة.

**تصميم التجربة:** أجريت الدراسة الحالية على ٤٠ من ذكور الجرذان البيضاء البالغة وزنها ٢٥٠ جم و تبلغ من العمر ٦ أشهر، تم تقسيمها إلى أربع مجموعات رئيسية (١٠ جرذان / لكل منها)؛ المجموعة الأولى هي مجموعة التحكم، المجموعة الثانية تلقت الجرذان الأكريلاميد داخل الصفاق، المجموعة الثالثة تلقت الأكريلاميد داخل الصفاق و السيلينيوم عن طريق الفم، المجموعة الرابعه تلقت السيلينيوم داخل الفم. أستمرت التجربة مدة ٤ أسابيع. وقد تم تخدير الجرذان ثم نحرها و استخراج عينة المخيخ وتجهيزها للفحص المجهري الضوئي والفحص المجهري الإلكتروني و القياسات المورفومترية.

**نتائج البحث:** أظهر الفحص المجهري الضوئي و الإلكتروني لأقسام المخيخ لجميع جرذان المجموعة الأولى صورة نسيجية مماثلة والتي كانت مطابقة للتركيب النسيجي الطبيعي للمخيخ. كما أظهر الفحص المجهري الضوئي للمجموعة الثانية؛ خلايا بركنجي مشوهة و ذات نوى متجمعة وألياف عصبية بيضاء منفصلة بشكل غير منتظم، و على النقيض ظهرت خلايا بركنجي في المجموعة الثالثة بشكل مماثل للخلايا في مجموعة التحكم مع وجود القليل من خلايا بركنجي المنكمشة. وقد أظهر الفحص المجهري الإلكتروني للمجموعة الثانية مجموعة من خلايا بركنجي المنكمشة ذات نوى داكنة مع عضيات حشوية مشوهة، وخلايا حبيبية متقلصة مقارنة بالمجموعة الثالثة، و التي لديها خلايا بركنجي و خلايا حبيبية ذات عضيات حشوية سليمة مع قليل من الخلايا ذات نوى متجانسة اللون ومخطط غير منتظم. كما أظهر التأثير المناعي لصبغة البروتين الحمضي الليفي الدبقي، رد فعل إيجابي قوي في المجموعة الثانية بالمقارنة برد الفعل الإيجابي المتوسط للمجموعة الثالثة، و الضعيف للمجموعة الرابعة.

**الخلاصة:** يقلل التناول المشترك للسيلينيوم مع مادة الأكريلاميد إلى حد كبير الآثار الضارة لمادة الأكريلاميد على مخيخ الفئران.