VER
Vascular & Endovascular Review

Possible Protective Role of Selenium Nanoparticles in Deltamethrin induced Cerebellar Toxicity in Adult Male Albino Rat: Histological, Immuno-histochemical and Biochemical Study

Dr. Asmaa kamel Hammad[1], Prof. Dr. Nabila Yousef Abd Elhalim[2], Dr. Reda Abd el Nasser Emam[3], Dr. Peter Hanna Eshak Yanni[4]

[1]Lecturer of Anatomy and Embryology Faculty of Medicine, Cairo University
[2]Professor of Anatomy and Embryology Faculty of Medicine, Cairo University
[3]Assistant Professor of Anatomy and Embryology Faculty of Medicine, Cairo University
[4]M.B., B.Ch., Demonstrator of Anatomy and Embryology Faculty of Medicine, Cairo University

ABSTRACT

Background: Deltamethrin administration induces neurotoxicity in the cerebellum through multiple mechanisms.

Aim of the Work: This study aimed to detect the adverse fluctuations occurring in the rat cerebellar cortex after deltamethrin administration. Furthermore, the possible ameliorative role of selenium nanoparticles to these changes was assessed.

Material and Methods: Forty adult male albinos' rats were divided into four groups (Gps): Gp I (Control), Gp II (sham), Gp III (Deltamethrin), Gp IV (Deltamethrin+ Selenium). Blood samples were taken by cardiac puncture for using in biochemical analyses. The cerebellum was processed for histological, immunohistochemical &biochemical analysis.

Results: The light microscopic analysis of deltamethrin group (III) revealed that the Purkinje cells were primarily affected by the neurotoxic effects of deltamethrin among the cerebellar cortex cells. In addition to demyelination of nerve fibers. A strong positive GFAP immunoreaction was observed in this group. On the other aspect, deltamethrin with selenium nanoparticles treated group (IV) showed obvious protection in the cerebellar histological architecture and weak positive GFAP immunoreaction. Biochemically, the cerebella of deltamethrin group (III) illustrated statistically significant increase in the level of MDA and a significant decrease in the level of GSH and the activity of SOD compared to statistically significant decrease in Gp IV (Deltamethrin+ Selenium).

Conclusion: In light of those observations, the current study revealed that selenium nanoparticles have protective role from the deleterious effects of deltamethrin on the cerebellar architecture, oxidative status and apoptosis.

KEYWORDS: Deltamethrin, cerebellum, Selenium nanoparticles.

How to Cite: Dr. Asmaa kamel Hammad, Prof. Dr. Nabila Yousef Abd Elhalim, Dr. Reda Abd el Nasser Emam, Dr. Peter Hanna Eshak Yanni, (2024) Possible Protective Role of Selenium Nanoparticles in Deltamethrin induced Cerebellar Toxicity in Adult Male Albino Rat: Histological, Immuno-histochemical and Biochemical Study, Vascular and Endovascular Review, Vol.7, No.2, 323-334

INTRODUCTION

One of the most well-known synthetic pyrethroids and the most effective neurotoxic insecticide is deltamethrin **(Petrovici et al., 2025)**. It has the ability to, directly and indirectly, disrupt the DNA structure and cellular antioxidant competence. Hepatotoxicity, nephrotoxicity, genotoxicity, immunosuppression, mutagenicity, and infertility are common health hazards of DLM toxicity **(Allam et al., 2022; Elbanna et al., 2023)**.

The cerebellum is concerned with maintaining balance, coordinating movements, as well as its function in learning and memory **(Bègue et al., 2024; Kim et al., 2024; McElroy et al., 2024).** Using elements like selenium (Se), a micronutrient and a cofactor of various antioxidant enzymes like thioredoxin reductase and glutathione peroxidase (GSH-Px), which maintain redox homeostasis, can minimize the harmful effect of deltamethrin **(Li et al., 2022; Moawad et al., 2024).**

Se deficiency can impair brain functions **(Zhang, 2023)**. It is now well recognized from various studies that Se can ameliorate the neurotoxic effect of many compounds **(Shehata et al., 2024; Zhang et al., 2023)**. However, there is a concern regarding the safety margin of Se and its bioavailability. So, nanotechnology science has been applied to overcome these limiting factors **(Skalickova et al., 2017).**

Although several studies have investigated the neuroprotective effects of selenium and selenium nanoparticles against deltamethrin-induced neurotoxicity, research specifically focusing on the cerebellar cortex in adult male albino rats remains limited.

Therefore, the present study had been designed to detect the adverse histological, biochemical and immune-histochemical changes occurring in the rat cerebellar cortex after deltamethrin administration. Furthermore, the possible protective role of selenium

nanoparticles to these changes was assessed**.**

Ethical Consideration

The Ethical Committee of Cairo University's Faculty of Medicine gave its approval to the project. According to the National Institutes of Health's rules for the care and use of laboratory animals, the study had been carried out in accordance with their ethical standards (NIH Publications No. 8023, revised 1978).

MATERIAL AND METHODS

Chemicals

Deltamethrin was obtained from Petsology Veterinary Clinic (Bab El Sharia, Cairo, Egypt) in the form of solution. It was prepared by mixing 0.5 g deltamethrin in 13.5 ml corn oil equivalent to 1 mg deltamethrin/ml corn oil **(Aziz et al., 2001)**. In addition, selenium nanoparticles solution was obtained from Nano Tech Egypt Company **(6th October, Giza, Egypt)**.

Animals

This study included forty male Sprague-Dawley albino rats weighing 170-200g. The animals were obtained from the Faculty of Medicine, Cairo University's animal house. They were kept in specialized metal cages. Standard rodent food pellets and sufficient water were provided to the animals.

Experimental design:

The rats were divided into four groups each one consisted of 10 rats as following:

Group I (Normal control): 10 rats received no medication. They were further subdivided into:

Group Ia: 5 rats that were sacrificed after 3 weeks.

Group Ib: 5 rats that were sacrificed after 6 weeks.

Group II (Sham Control): 10 rats received intra peritoneal (I.P) corn oil (the vehicle) injection (1 ml) daily and further subdivided into:

Group IIa: 5 rats that were sacrificed after 3 weeks.

Group IIb: 5 rats that were sacrificed after 6 weeks.

Group III: 10 rats received deltamethrin dissolved in corn oil at a dose of 12.5 ml/kg/day I.P for 3 weeks **(Wu and Liu, 2000)** then they were subdivided into:

Group IIIa (deltamethrin group): 5 rats that were sacrificed after3weeks.

Group IIIb (recovery group): 5 rats that left for another 3 weeks without administration of deltamethrin then sacrificed after 6 weeks.

Group IV (deltamethrin +selenium nanoparticles): 10 rats were subdivided into:

Group IV a: 5 rats received deltamethrin dissolved in corn oil at a dose of 12.5 ml/kg/day I.P and selenium nanoparticles 0.5 ml/kg/ day I.P concomitantly and sacrificed after 3 weeks **(Abou Zaid et al., 2017).**

Group IV b: 5 rats received deltamethrin dissolved in corn oil at a dose of 12.5 ml/kg/day I.P and selenium nanoparticles 0.5 ml /kg/day I.P for 3 weeks concomitantly then continuation of SeNPS alone for another 3 weeks and sacrificed after 6 weeks.

By the end of the experiment:

Light microscopic study: The animals were anesthetized by inhaling ether and then sacrificed. The cerebellum was separated carefully, fixation was done by 10% formalin and five micrometers (μm) thick paraffin sections were prepared for the following:

1-Hematoxylin and Eosin & Bielschowsky silver staining to determine the cerebellar architecture.

2-Immunohistochemical study: a-Glial Fibrillary Acidic Protein (GFAP) for detection of astrocyte: Specimens were examined by light microscopy and photographed at ×400 & ×1000 magnification.

Histomorphometric study: Image J analysis software was used to carry out the quantitative study. The specimen preparations of the cerebellum from each rat were subjected to quantitative studies in 10 non-overlapping microscopic fields picked randomly from each slide. The standard measuring frame of a recognized area measuring 11694.91 m2 was used to assess them. Those measured parameters were:

1-The number of Purkinje cells per each folium counted in H&E-stained sections under magnification 100.

2-The mean optical density of axons myelination by silver staining under magnification 1000.

3- The area percent of GFAP immune-reactivity in the cerebellar cortex under magnification 400.

Biochemical study: Cardiac punctures were used to obtain blood samples for biochemical analysis.

A- **Oxidative stress marker**: Malondialdhyde (MDA), a byproduct of lipid peroxidation, is used to assess the degree of oxidative stress in a tissue **(Onaolapo et al., 2017)**.

B- **Antioxidative stress marker (Onaolapo et al., 2017):** The methods were carried out in following the guidelines provided by the manufacturer. Using a commercial kit (Biodiagnostics), the inhibition of nitroblue tetrazolium reduction by O2-generated by the xanthine/xanthine oxidase system was used to determine the superoxide dismutase (SOD) activity in tissue homogenate. The reduction of dithiobis (2-nitrobenzoic acid) (DTNB) with reduced glutathione to produce a yellow compound is the base for the amount of the tissue level of reduced glutathione (GSH).

C- **Measurement of serum serotonin and glutamate:** The serum serotonin levels were measured using a commercial enzyme immunoassay kit (Immuno-Biological Laboratories, Inc. - IBL - Minneapolis, USA). Serum glutamate

concentration was measured by means of Glutamate Assay Kit (Abnova, Taipei City,Taiwan) following the manufacturer's protocol specifications. The result was expressed in NG/ML of blood sample **(El-Beltagy et al., 2019)**.

Statistical analysis:
Numerical data of the histomorphometric measurements and the biochemical levels were analyzed using the Statistical Package for Social Science (SPSS) version 22. Results were presented as mean ± standard deviation (SD). Statistical evaluation was done using one-way analysis of variance (ANOVA) followed by Bonferroni pairwise comparisons. The quantitative data were examined by Kolmogorov Smirnov test for normality. Significance was considered when the P value was ≤ 0.05.

RESULTS

Histological results:
Light microscopic results:
Histological examination of cerebellar sections of control rats' group I and sham group II by H&E showed the classic architecture appearance of cerebellar cortex which was composed of 3 layers. The molecular layer was the outermost; formed of superficially located spindle-shaped stellate cells and deeper located rounded basket cells. The Purkinje layer consists of monolayer continuous large pyriform somata of Purkinje neurons with clear centrally located vesicular nuclei, prominent nucleoli and was surrounded by eosinophilic cytoplasm containing basophilic Nissl granules.

The apical dendrites were also detected with its arborization. The granular layer was formed of tightly packed small rounded basophilic cells with darkly stained nuclei and non-cellular (cerebellar islands) in between. Bergmann astrocytes with distinctive open-faced nuclei were also noticed. (Figs:3&4)

Light microscopic examination of sections of group (III) revealed widespread neuronal affection specifically of the Purkinje cell layer which was reflected on the other two layers. The Purkinje cells revealed disturbed normal linear organization with marked disarrangement being disposed in more than one row. Marked loss of Purkinje cells was observed. The Purkinje cells appeared shrunken and distorted with patchy loss of Purkinje cells leaving empty spaces associated with neuron degeneration in the form of an eosinophilic cytoplasm and small dark pyknotic nuclei surrounded by many vacuolations in addition to migration of Purkinje cells in the granular cell layer. Many apoptotic Purkinje cells with peripherally situated nuclei and dispersal of Nissl bodies, fragmented nuclei with chromatin irregularly distributed throughout the cytoplasm and also degenerated swollen Purkinje cell with eosinophilic cytoplasm and loss of its nucleus (Ghost cell) are detected (Fig:5).

Light microscopic examination of sections of group (IV) showed Purkinje cells arranged in linear organization. Most of Purkinje cells appeared with central rounded nuclei, prominent nucleoli and apical dendrite with some vacuolated areas around them. Some Purkinje cells showed ill-defined nucleus and cytoplasmic structures. (Fig. 6&7).

The silver-stained sections in group I showed the classic three layers of cerebellar cortex. The pyriform Purkinje cells with stained cell body, prominent apical dendrite and clear axon. The nuclei appeared centrally-located and prominent. The nerve fibers had a straight course and showed a pale-staining appearance (Fig:1). The results of group II were similar to group I (Fig:2). The silver-stained sections in group III showed shrunken, distorted Purkinje cells with argyrophilic cytoplasm. Some nerve fibers were thickened and spitted. (Fig. 8).

The silver-stained sections of group IV showed pyriform pale-stained Purkinje cells with preserved linear arrangement. The Purkinje cells showed clear nucleus, prominent nucleolus. Thickened nerve fibers were also noted in the Purkinje and molecular layers. Some pericellular unstained haloes in Purkinje and molecular cell layers were detected (Fig. 9).

There were no differences between the rats' sacrifice 3 weeks and those of the 7 weeks so the time zone existing was that of 7 weeks.

Immuno-histochemical results:
1-GFAP immune-reactivity:
GFAP stained sections in group I and II, which is specific to detect astrogliosis, revealed normal immune-reactivity in all cerebellar cortical layers **(Figs. 10&11)**.
GFAP stained sections in group III induced by deltamethrin showed increased GFAP immune reaction in different cerebellar cortical layers **(Fig.12)**.
GFAP stained sections in group IV induced by deltamethrin with selenium nanoparticles revealed mild GFAP immune reactivity in different cerebellar cortical layers **(Fig.13)**.

Histomorphometric studies of the mean area percent of GFAP, which is specific to detect astrogliosis, showed statistically significant increase in deltamethrin group (III) compared to control and sham groups. In the concomitant deltamethrin and selenium nanoparticles treated group (IV), there was a statistically significant decrease in the mean area percent of GFAP immune reactivity in relation to the deltamethrin group and slightly increase in relation to control and sham groups.

Biochemically,
Compared with the control I and sham II groups, the Purkinje cells in the deltamethrin group (III) were significantly reduced in number. The Purkinje cells in group (IV) increased in number compared to the deltamethrin group. The recovery group (III)

showed decrease in the number of Purkinje cells compared to the control and sham groups. Table (1).
Mean value of area percentage for GFAP immune- reactivity in the cerebellar cortex in the different rat groups showed increase in deltamethrin group (III) compared to the control group (I) and sham group (II). Meanwhile, group (IV) revealed decrease in immune- reactivity of GFAP compared to deltamethrin group and recovery group and slightly increased compared to control group. Table (2).

The cerebella of deltamethrin group (III) illustrated statistically significant increase in the level of MDA and a significant decrease in the level of GSH and the activity of SOD compared to control and sham groups. In concomitant deltamethrin and selenium nanoparticles treated group (IV), there was a statistically significant decrease in the level of MDA compared to the deltamethrin group and slightly significant increase compared to control and sham groups and increase in the level of GSH and the activity of SOD compared to the deltamethrin group and slightly decrease compared to control and sham groups Tables (3&4&5).

Also, the serum serotonin and glutamate levels in deltamethrin group (III) showed statistically significant decrease compared to control and sham groups. But there was statistically significant increase in serum serotonin and glutamate levels in concomitant deltamethrin and selenium nanoparticles treated group (IV) compared to deltamethrin group and slightly significant decrease compared to control and sham groups. Table (6&7).

The deltamethrin group (III) showed decrease in axons myelination in comparison to control and sham groups. In SeNPs and deltamethrin concomitant group (IV) showed increase in axons myelination compared to the deltamethrin group and slightly decrease compared to control group. Table (8).

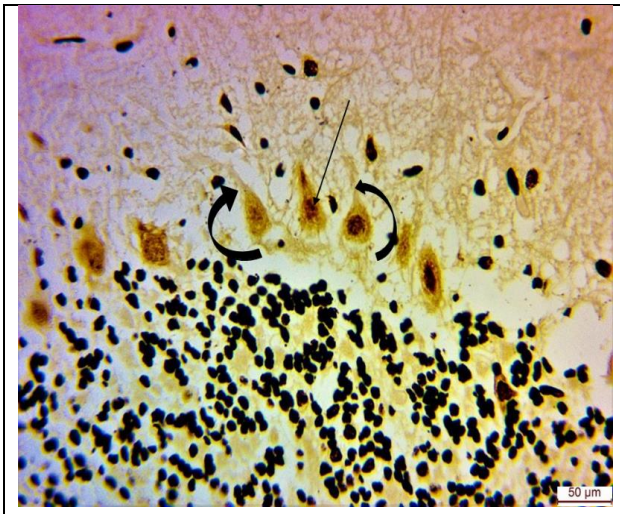


Fig. (1): A photomicrograph of a section of rat cerebellum in control group (I) showing that Purkinje cells are pyriform in shape cells being arranged in a single layer with their axons (curved arrows) and cell body that contains nucleus, and prominent nucleolus (straight arrow).

(Silver stain x400)

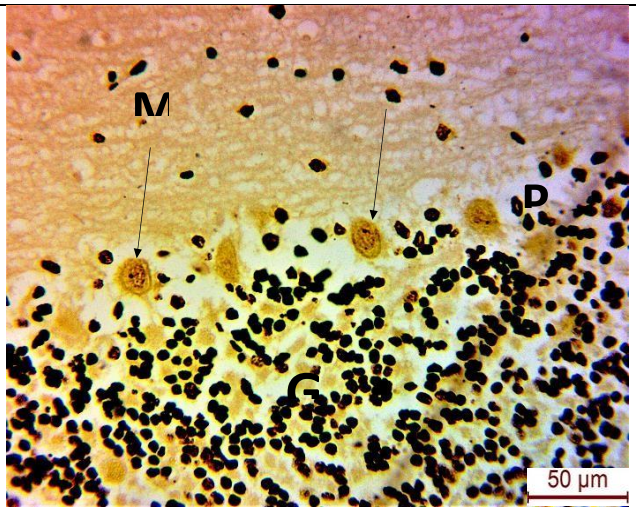


Fig. (2): A photomicrograph of a section of rat cerebellum in sham control group (II) showing Purkinje layer (PL) in a single layer between the molecular (ML) and granular (GL) layers. The Purkinje cells (arrows) show clear nucleus with prominent nucleolus

(Silver stain x400)

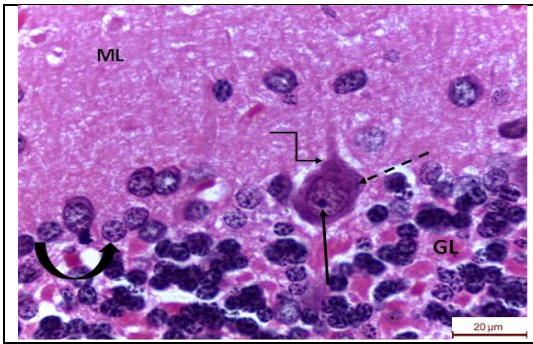


Fig. (3): A photomicrograph of a section in the cerebellar cortex of a rat of the control group (I) showing large pyriform Purkinje cell with large central vesicular nuclei with prominent nucleoli (arrow) and

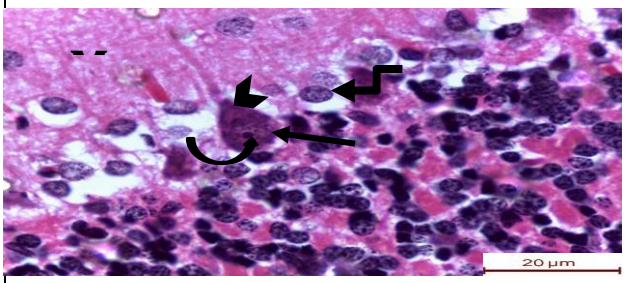
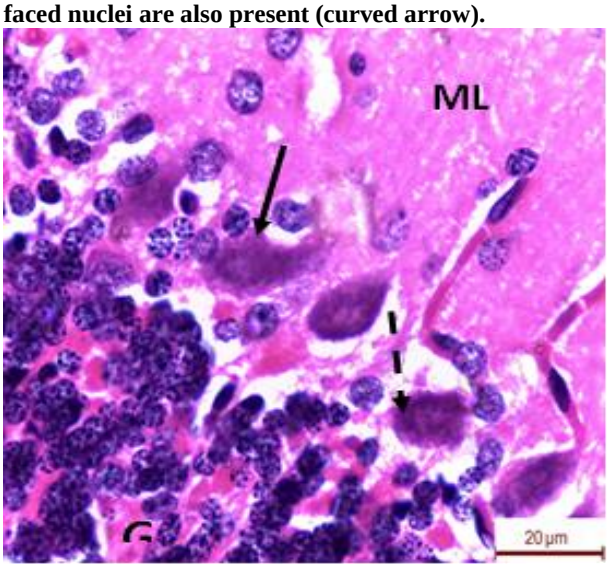


Fig. (**4**): A photomicrograph of a section in rat cerebellum from sham group (II) showing flask-shaped appearance of the Purkinje cell with centrally located vesicular nucleus (arrow) and prominent nucleolus(curved arrow) .The arrow head points to the apical

basophilic Nissl granules in cytoplasm (dotted arrow).The beginning of dendritic arbor of Purkinje cell (zigzag arrow). Bergmann astrocytes with typical open-faced nuclei are also present (curved arrow).



g. (5): A photomicrograph of a section in the cerebellar rtex of a rat of the deltamethrin group (III a) showing a generated swollen Purkinje cell with loss of its nucleus host cell) (arrow). Other Purkinje cell showed periphery rk fragmented nucleus (Karyorrhexis) with loss of apical ndrites (dotted arrow) .

dendrites of Purkinje cell (arborization). Bergmann astrocytes with typical open-faced nuclei are also present (zigzag arrow).

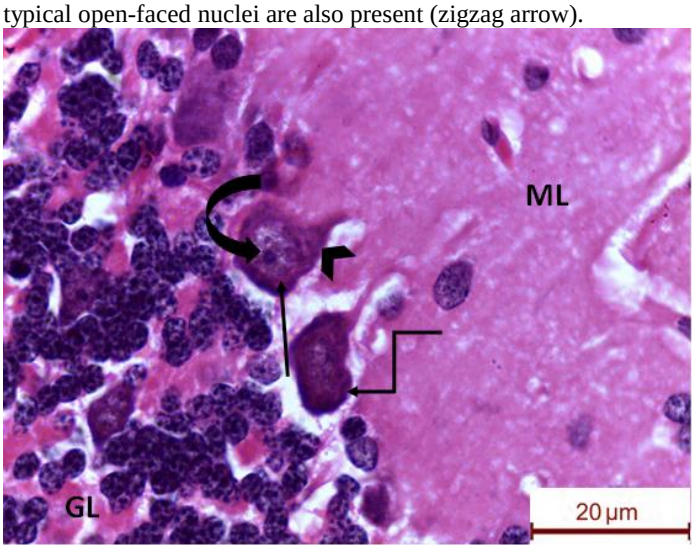


Fig. (6): A photomicrograph of a section in the cerebellar cortex of a rat of the deltamethrin + SeNPs group (IV) showing Purkinje cell with vesicular nucleus (arrow) and prominent nucleolus (curved arrow) surrounded by vacuolated cytoplasm with apical dendrite (arrow head). Another Purkinje cell appeared with ill-defined nuclei and cytoplasm (zigzag arrow).

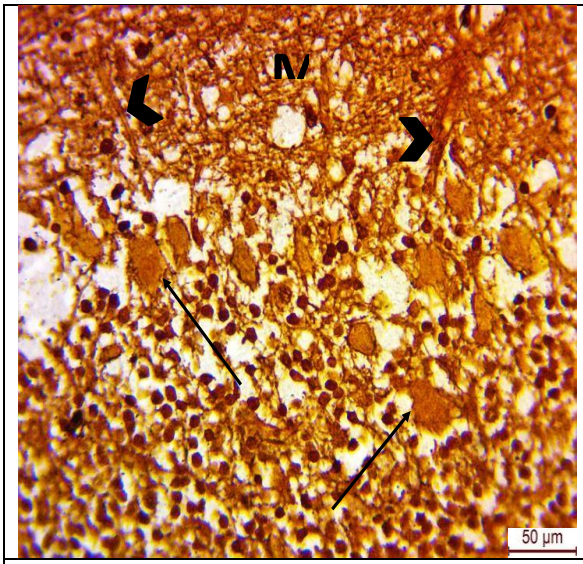


Fig. (8): A photomicrograph of a section in rat cerebellum from deltamethrin group (III) showing loss of linear organization of purkinje cells being disposed in more than one row with darkly stained pyknotic nuclei (curved arrows) with loss of apical dendrites. Marked vacuolation is observed (arrows) in the molecular layer (ML), Pukinje layer (PL) and granular layer (GL). (H&E x400)

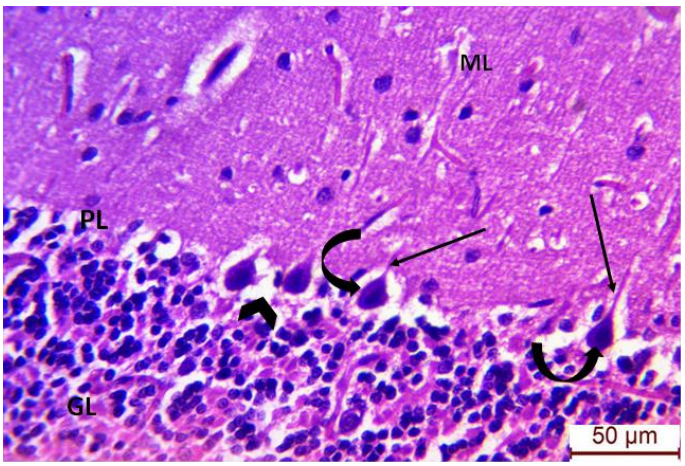


Fig. (7): A photomicrograph of a section in rat cerebellum from deltamethrin + SeNPs group (IV) showing flask shaped Purkinje cells in linear arrangement (PL) with central rounded nuclei and prominent nucleolus (curved arrows) with preserved apical dendrites (arrows). Minimal vacuolation (arrow head) is observed around the Purkinje cells. (H&E x400)

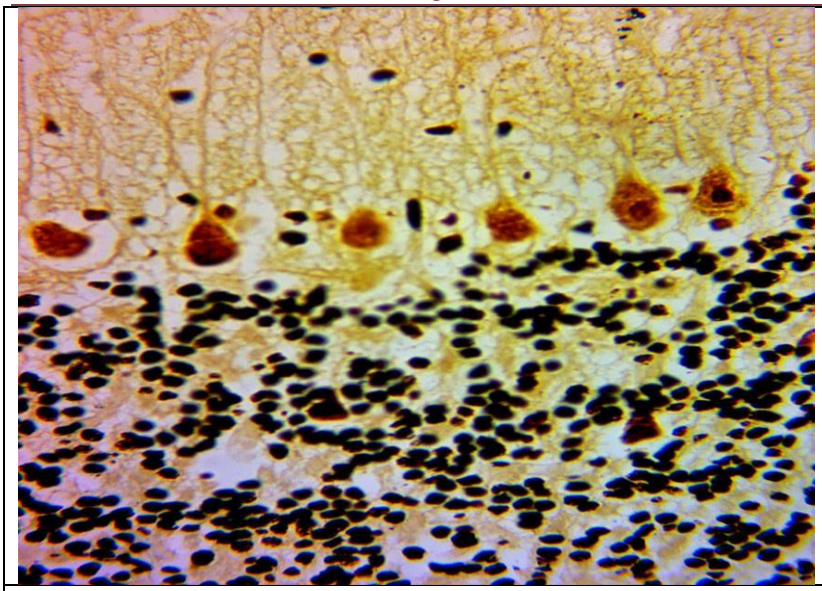


Fig. (9) A photomicrograph of a section in rat cerebellum from deltamethrin + SeNPs group (IVa) showing preserved linear arrangement of purkinje cells with clear nucleus and prominent nucleolus (dotted arrow). Thickened nerve fibers are also noted in the purkinje and molecular layers (black arrows). some pericellular unstained haloes in Purkinje and molecular cell layers (arrow heads) are noted.

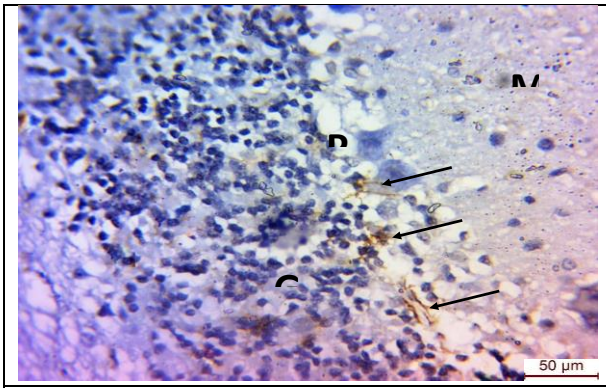


Fig. (10): A photomicrograph of a section of the cerebellar cortex of a rat in the control group (I) showing normal glial fibrillary acidic protein (GFAP) immune reaction for astrocytes in the Purkinje layer (PL) and granular layer (GL) (arrows).

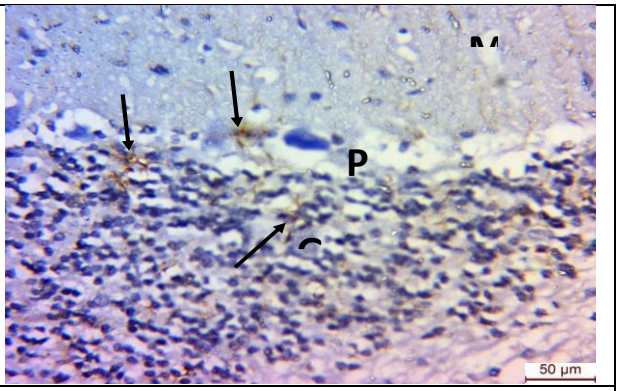


Fig. (11): A photomicrograph of a section of the cerebellar cortex of a rat in the sham control group (II) showing normal glial fibrillary acidic protein (GFAP) immune reaction for astrocytes (arrows) in the Purkinje layer (PL) and granular layer (GL).

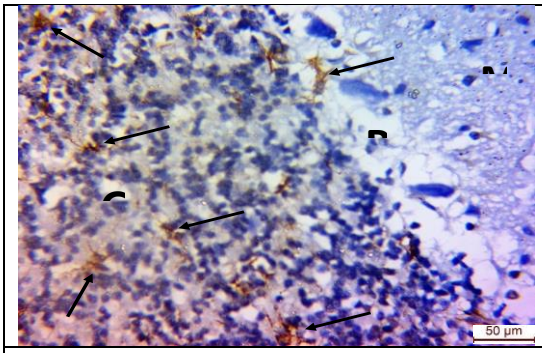


Fig. (12): A photomicrograph of a section of the cerebellar cortex of a rat in the deltamethrin group (III) showing moderate GFAP immune reaction for astrocytes (arrows) in the Purkinje layer (PL) and granular layer (GL).

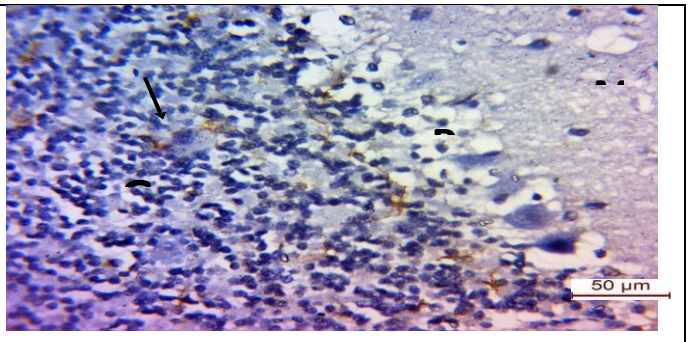


Fig. (13): A photomicrograph of a section of the cerebellar cortex of a rat in the deltamethrin + SeNPs group (IV) showing mild GFAP immune reaction for astrocytes (arrows) in the granular layer (GL).

Table (1): The mean number of Purkinje cells/each folium among the different groups.

Groups	Range	Mean ± SD	*P* - value
Ia	35.68 - 41.48	**38.58 ± 2.90**	**.000***
Ib	29.24 - 40.56	**34.90 ± 5.66**	**.000***
IIa	32.93 - 39.83	**36.38 ± 3.45**	**.000***
IIb	33.14 - 40.18	**36.66 ± 3.52**	**.000***
IIIa	13.66 - 19.1	**16.38 ± 2.72**	**.000***
IIIb	15.67 - 21.29	**18.48 ± 2.81**	**.000***
IVa	23.77 - 27.83	**25.80 ± 2.03**	**.000***
IVb	25.08 - 31.16	**28.12 ± 3.04**	**.001***

Using ANOVA followed by Bonferroni pairwise comparisons

* : Statistically significant P value ≤ 0.05

Table (2): The mean area percent of GFAP immune -reactivity among the different groups

Groups	Range	Mean ± SD	*P* - value
Ia	**18.04 – 22.12**	**20.08 ± 2.04**	**0.000***
Ib	**15.94 – 20.82**	**18.38 ± 2.44**	**0.000***
IIa	**15.41 – 19.99**	**17.70 ± 2.29**	**0.000***
IIb	**14.73 – 19.67**	**17.20 ± 2.47**	**0.000***
IIIa	**47.62 – 53.94**	**50.78 ± 3.16**	**0.000***
IIIb	**40.37 – 46.07**	**43.22 ± 2.85**	**0.000***
IVa	**23.59 – 29.65**	**26.62 ± 3.03**	**0.016***
IVb	**20.97 – 27.15**	**24.06 ± 3.09**	**0.735**

Using ANOVA followed by Bonferroni pairwise comparisons

* : Statistically significant P value ≤ 0.05

Table (3): The mean value of malondialdhyde (MDA) level among the different groups

Groups	Range	Mean ± SD	*P* - value
Ia	7.44 – 9.24	**8.34 ± 0.9**	**.000***
Ib	6.76 – 9.56	**8.16 ± 1.4**	**.000***
IIa	7.777 – 7.86	**7.82 ± .043**	**.000***
IIb	7.32 – 8.4	**7.86 ± 0.54**	**.000***
IIIa	60.56 – 63.92	**62.24 ± 1.68**	**.000***
IIIb	56.67 – 59.05	**57.86 ± 1.19**	**.000***
IVa	24.23 – 26.13	**25.18 ± 0.95**	**.000***
IVb	19.52 – 21.8	**20.66 ± 1.14**	**.000***

Using ANOVA followed by Bonferroni pairwise comparisons

* : Statistically significant P value ≤ 0.05

Table (4): The mean value of superoxide dismutase (SOD) activity among different groups

Groups	Range	Mean ± SD	*P* - value
Ia	3.57 – 4.79	**4.18 ± 0.61**	**.000***
Ib	3.61 – 4.63	**4.12 ± 0.51**	**.000***
IIa	3.76 – 5.04	**4.40 ± 0.64**	**.000***
IIb	3.83 – 5.17	**4.50 ± 0.67**	**.000***
IIIa	0.69 – 1.83	**1.26 ± 0.57**	**.000***
IIIb	0.83 – 2.01	**1.42 ± 0.59**	**.000***
IVa	2.37 – 3.51	**2.94 ± 0.57**	**.066**
IVb	2.59 – 3.73	**3.16 ± 0.57**	**.294**

Using ANOVA followed by Bonferroni pairwise comparisons

* : Statistically significant P value ≤ 0.05

Table (5): The mean value of reduced glutathione (GSH) level among different groups

Groups	Range	Mean ± SD	*P* - value
Ia	72.76 – 80.16	**76.46 ± 3.70**	**.000***
Ib	73.66 – 81.46	**77.56 ± 3.90**	**.000***
IIa	74.66 – 83.46	**79.06 ± 4.40**	**.000***
IIb	74.04 – 82.2	**78.12 ± 4.08**	**.000***
IIIa	33.95 – 40.49	**37.22 ± 3.27**	**.000***
IIIb	36.01 – 42.11	**39.06 ± 3.05**	**.000***
IVa	51.2 – 58.04	**54.62 ± 3.42**	**.000***
IVb	53.64 – 62.24	**57.94 ± 4.30**	**.000***

Using ANOVA followed by Bonferroni pairwise comparisons

* : Statistically significant P value ≤ 0.05

Table (6): The mean value of serotonin level among different groups

Groups	Range	Mean ± SD	*P* - value
Ia	32.43 – 36.81	**34.62 ± 2.19**	**.000***
Ib	29.04 – 36.0	**32.52 ± 3.48**	**.000***
IIa	34.15 – 41.21	**37.68 ± 3.53**	**.000***
IIb	34.3 – 41.62	**37.96 ± 3.66**	**.000***
IIIa	11.81 – 15.75	**13.78 ± 1.97**	**.000***
IIIb	13.38 – 17.94	**15.66 ± 2.28**	**.000***
IVa	21.65 – 25.91	**23.78 ± 2.13**	**.000***
IVb	23.45 – 29.95	**26.70 ± 3.25**	**.004***

Using ANOVA followed by Bonferroni pairwise comparisons

* : Statistically significant P value ≤ 0.05

Table (7): The mean value of glutamate level among different groups

Groups	Range	Mean ± SD	*P* - value
Ia	24.5 – 28.1	**26.30 ± 1.80**	**0.000***
Ib	22.67 – 25.93	**24.30 ± 1.63**	**0.000***
IIa	25.75 – 30.37	**28.06 ± 2.31**	**0.000***
IIb	27.64 – 32.04	**29.84 ± 2.20**	**0.000***
IIIa	10.97 – 15.59	**13.28 ± 2.31**	**0.000***
IIIb	11.62 – 17.34	**14.48 ± 2.86**	**0.000***
IVa	16.89 – 19.99	**18.44 ± 1.55**	**0.000***
IVb	17.4 – 21.72	**19.56 ± 2.16**	**0.001***

Using ANOVA followed by Bonferroni pairwise comparisons

* : Statistically significant P value ≤ 0.05

Table (8): The mean optical density of axons myelination by silver staining among the different groups.

Groups	Mean ± SD	*P* – value
Ia	**185.8 ± 3.49**	**0.000***
Ib	**177.2 ± 3.96**	**0.000***
IIa	**173.2 ± 3.49**	**0.000***
IIb	**175 ± 3.8**	**0.000***
IIIa	**126.6 ± 4.39**	**0.000***
IIIb	**123.8 ± 3.83**	**0.000***
IVa	**160.4 ± 3.84**	**0.000***
IVb	**160 ± 3.16**	**0.001***

Using ANOVA followed by Bonferroni pairwise comparisons

* : Statistically significant P value ≤ 0.05

DISCUSSION

Deltamethrin is considered one of the most potent and widely used insecticide. But, exposure to deltamethrin contaminated air, water and food is a source of its neurotoxicity in humans **(El-Beltagy et al., 2019)**. Previous studies have shown that Se NPs can reduce the harmful effects of various chemicals on various human organs like the brain, liver and kidney **(Ali et al., 2020)**. It is commonly known that the cerebellum plays an essential role in memory, learning, balance, and impulse control. So, the cerebellum was chosen for the present study **(Tedesco et al., 2011)**.

The present study demonstrated neuroprotective role of SeNPs in deltamethrin-induced cerebellum toxicity of adult male albino rats through various mechanisms as they had antioxidative and anti-inflammatory activities **(Surai, 2002)**. In the current work, deltamethrin has induced disturbance in the histological structure of the cerebellar cortex in deltamethrin group (III), particularly in the Purkinje cell layer more than the molecular and granular layers, with the Purkinje cells arranged in multiple layers rather than monolayer. This was matched with the results of **(Kassab, 2018),** which might be explained by the prolongation of neuronal insult as an adaptive mechanism in the form of crowding of Purkinje cells, in a trial to reestablish synapsis with the other nerve cells to be able to achieve their functions **(El-Dien et al., 2010)**. Shrunken Purkinje cells with pyknotic nuclei and dark-stained cytoplasm were also observed in deltamethrin group (IIIa). This was confirmed by **(Wu & Liu, 2000),** and attributed to neurons degeneration **(Garman, 2011).** In addition, loss of Nissl bodies and swollen Purkinje cells were noticed. Similar findings were recorded in diabetic rats and were referred to the apoptosis of Purkinje neurons and neuroglia **(Mohamed, 2012)**. In the present study, migration of some Purkinje cells into the granular layer was observed in deltamethrin group (III) and this could be explained as a defect in cell signaling, transport and energy metabolism **(Rösen et al., 2001)**. Furthermore, Purkinje layer vacuolations recorded in the current work in rats treated with deltamethrin group (III), and these results run in alignment with **(Ogaly et al., 2015)** results which could be clarefied by either swelling or degeneration of neuronal and astrocytic processes or myelin sheath splitting and edema **(Garman, 2011)**.

Another finding encountered in the deltamethrin group (III) was the presence of dilated congested blood capillaries. This finding was in agreement with other researchers who explained it on the basis of compromising the vascular endothelial cells, leading to the release of reactive oxygen species which are regarded as important endothelial relaxing factors. This also leads to production of significant cytotoxicity and release of cytokines and other inflammatory mediators that increase the permeability of the blood-brain barrier **(Hussein et al., 2018)**. Also, abnormal apoptotic granule cells with vacuolations appeared in the granular layer of the cerebellar cortex of rats treated with deltamethrin group (III). This comes in contact with other authors who attributed it to the secondary changes in the Purkinje cells when they failed to establish contact with the granule cells, leading to a lack of normal synchronism between them **(Kumar et al., 2013)**. Prominent peri-neuronal spaces and vacuolations around both basket and stellate cells in the molecular layer of the cerebellar cortex of the deltamethrin group (III) might be referred to the shrinkage of cells and disintegration of cytoskeletal elements that resulted from disruption of the tight junction in the rat blood-brain barrier **(Kassab, 2018)**.

With silver staining, the Purkinje cells of deltamethrin group (III) aappeared darkly-stained and exhibited irregular outlines and strongly argyrophilic cytoplasm. Moreover, the significant decline in the optical density implied its demyelinating neurotoxic changes. This demyelinating effect might be attributed to the changes in myelin basic protein leading to membrane damage and axonal degeneration **(Afifi, 2009)**. The latter author also suggested that intra-myelinic edema and separation of myelin lamellae between the injured neurons disable maintaining their distal processes being part of a ‘dying back’ process of neuronal injury. Although the previous study was done on sodium fluoride-induced cerebellar injury and no available literature discussed the demyelinating impact of deltamethrin, further research is deserved to elucidate the mechanism beyond this impact. GFAP is a specific marker for detection of astrogliosis. Reactive astrogliosis is an end result of changes that occur in response to all CNS insults, triggered by particular signaling events and these changes depend on the degree of the insult. Changes in tissue architecture can be either permanent or reversible. So, reactive astrogliosis and scar formation have been the primary cause of CNS diseases. As a result, reactive astrocytes are considered a potential target for cutting-edge therapeutic approaches for a range of CNS diseases **(Pekny & Pekna, 2014)**.

According to **(Bates et al., 2002),** Neurotoxic substances are produced by astrocytes like inflammatory cytokines and free radicals during reactive astrogliosis. This causes neuronal damage and participates in the progression of neurodegenerative disorders. Therefore, elevated GFAP expression serves as a sensitive and precise indicator of disease- and toxin-induced brain damage **(Hol & Pekny, 2015)**. In the current study, increased immune reactivity of GFAP was detected in all cerebellar cortical layers in deltamethrin group (III) compared to control and sham control groups. In addition, there was statistically significant increase of the mean area percent of GFAP immune-reactivity in the morphometric results of the same groups. This was matched with **(Khalil et al., 2022)** who illustrated the toxic effect of deltamethrin.

The brain is highly sensitive to reactive oxygen species (ROS) due to its high oxygen uptake, rich polyunsaturated fatty acid content of the neuronal membranes, weak antioxidant defenses and several auto-oxidizable neurotransmitters. So, the neural network is particularly susceptible to damage from ROS **(Patel, 2016)**. Lipid peroxidation is a marker of oxidative damage and MDA is a stable end product of lipid peroxidation that can be used as an indirect measure of cumulative lipid peroxidation **(Salim et al., 2016)**. Moreover, GSH is the major endogenous antioxidant scavenger that protects the cells from oxidative stress due to its capacity to bind to ROS and scavenge them **(Habib et al., 2007)**. In the present study, significant changes in MDA, SOD and GSH levels were observed in deltamethrin group (III) in the form of increased MDA levels and decreased SOD and GSH levels.

These findings were in alignment with **(Lu et al., 2019)** who clarified the oxidative stress caused by deltamethrin. Serotonin or 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter critical for controlling various behavioral and physiological processes including mood, sleep, appetite, aggression and sexual behavior. Serotonin regulates synaptic transmission, neuronal activity and cerebellar growth **(Young, 2007)**. In the current study, significant decrease in serum serotonin was detected in deltamethrin group (III) and it was similar to results of **(El-Beltagy et al., 2019)**. This was explained by the potential role of deltamethrin in stimulation of calcium influx in cerebellar neurons through prolongation of the opening time of calcium channels **(Pitzer et al., 2021)**.

Regarding glutamate, The, anion form of glutamic acid, that is important for signal neurotransmission in some areas of the brain, especially in the Purkinje and granular cells of the cerebellum. Glutamate plays a critical role in the regulation of synaptogenesis during early brain development **(Meldrum, 2000).** Decreased serum glutamate levels were detected in deltamethrin group (III) which was augmented by results of **(El-Beltagy et al., 2019).** This was explained as the loss of Purkinje cells leads to reduction in glutamate release in cerebellar efferent pathways **(McKimm et al., 2014)**. Administration of SeNPs ameliorates deltamethrin neurotoxicity and this appeared in preservation of the histological architecture pattern of the cerebellar cortex in concomitant deltamethrin and SeNPs group (IV) in the form of regular arrangement of Purkinje cells with maintenance of their flask shape appearance, reduction of the cytoplasmic vacuolations and decrease of darkly-stained nuclei. In addition, the morphometric results of the present study displayed that the mean area percent of purkinje cell/folium in the concomitant deltamethrin and SeNPs group (IV) showed statistically-significant increase in comparison to deltamethrin and recovery groups and slightly decrease to control and sham groups. These findings come in contact with **(Khalil et al., 2022)** who pointed out the effect of SeNPs in improving several cerebellar histopathological lesions by their radical scavenging and antioxidant effects. With silver staining, the concomitant deltamethrin and SeNPs group (IV) showed preserved architecture appearance of cerebellar cortical layers and increase in the optical density of some nerve fibers thickening. This effect might be attributed to the nerve regeneration **(Turgut et al., 2005)**.

Furthermore, Mild immune reactivity of GFAP and decrease the mean area percent of GFAP immune-reactivity in concomitant deltamethrin and SeNPs group (IV) were observed in the present study in comparison to deltamethrin and recovery groups and slightly increase to control and sham groups. This comes in contact with **(Khalil et al., 2022)**. In addition, Selenium also acts as a cofactor in the formation of selenoproteins involved in the catalysis of hydroperoxide and removal ROS products **(Dawood et al., 2021).** Decrease in MDA level and increase in SOD activity and GSH level were observed in rats of concomitant deltamethrin and SeNPs group (IV). These findings run parallel with results of **(Al Kahtani, 2020)** who pointed to the ameliorative effect of SeNPs against oxidative stress. A significant increase of serum serotonin and glutamate was demonstrated in concomitant deltamethrin and SeNPs group (IV) compared to deltamethrin and recovery groups and slightly decrease to control and sham groups. This was matched with **(Al Kahtani, 2020; Al Omairi et al., 2022)** who elucidated that SeNPs compensated the lack of adequate amounts of serum serotonin which is considered as one of the most essential neurotransmitters that affects the central and peripheral nervous systems. So, SeNPs have the ability to improve cognitive function by modulating the neurotransmitter function in the brain. However, the mechanism of SeNPs on glutamate are still unclear so, further researches are deserved to illustrate the explanation beyond this impact.

CONCLUSION

Deltamethrin can induce neurotoxicity through oxidative stress by generation of reactive oxygen species which initiate apoptosis. Selenium nanoparticles can protect the cerebellum from the changes occurred by deltamethrin through their antioxidant and anti-apoptotic effects. Although Selenium nanoparticles have protective effect from the toxicity of deltamethrin that detected on the histological, immuno-histochemical and biochemical levels.

Financial Support
None

Conflict Of Interests
There are no conflicts of interest. The paper isn't published in other journals or presented at a meeting, organization, or any other place

REFERENCES

1. Abou Zaid, O. A. R., El-Sonbaty, S. M., & Barakat, W. (2017). Ameliorative effect of selenium nanoparticles and ferulic acid on acrylamide-induced neurotoxicity in rats. Ann. Med. Biomed. Sci, 3(2), 35–45.
2. Afifi, O. K. (2009). Effect of sodium fluoride on the cerebellar cortex of adult albino rats and the possible protective role of vitamin B6: A light and electron microscopic study. Egypt J Histol, 32(2), 358–367.
3. Al Kahtani, M. (2020). Effect of both selenium and biosynthesized nanoselenium particles on cadmium-induced neurotoxicity in albino rats. Human & experimental toxicology, 39(2), 159–172.
4. Al Omairi, N. E., Albrakati, A., Alsharif, K. F., Almalki, A. S., Alsanie, W., Abd Elmageed, Z. Y., Zaafar, D., Lokman, M. S., Bauomy, A. A., & Belal, S. K. (2022). Selenium nanoparticles with prodigiosin rescue hippocampal damage associated with epileptic seizures induced by pentylenetetrazole in rats. Biology, 11(3), 354.
5. Ali, H. F., El-Sayed, N. M., Ahmed, A. A., Hanna, P. A., & Moustafa, Y. M. (2020). Nano selenium ameliorates oxidative stress and inflammatory response associated with cypermethrin-induced neurotoxicity in rats. Ecotoxicology and environmental safety, 195, 110479.

6. Allam, A., Abdeen, A., Devkota, H. P., Ibrahim, S. S., Youssef, G., Soliman, A., Abdel-Daim, M. M., Alzahrani, K. J., Shoghy, K., & Ibrahim, S. F. (2022). N-acetylcysteine alleviated the deltamethrin-induced oxidative cascade and apoptosis in liver and kidney tissues. International journal of environmental research and public health, 19(2), 638.
7. Aziz, M. H., Agrawal, A. K., Adhami, V. M., Shukla, Y., & Seth, P. K. (2001). Neurodevelopmental consequences of gestational exposure (GD14–GD20) to low dose deltamethrin in rats. Neuroscience letters, 300(3), 161–165.
8. Bates, K., Fonte, J., Robertson, T., Martins, R., & Harvey, A. (2002). Chronic gliosis triggers Alzheimer's disease-like processing of amyloid precursor protein. Neuroscience, 113(4), 785–796.
9. Bègue, I., Elandaloussi, Y., Delavari, F., Cao, H., Moussa-Tooks, A., Roser, M., Coupé, P., Leboyer, M., Kaiser, S., & Houenou, J. (2024). The cerebellum and cognitive function: anatomical evidence from a Transdiagnostic sample. The Cerebellum, 23(4), 1399–1410.
10. Dawood, M. A., Basuini, M. F. E., Yilmaz, S., Abdel-Latif, H. M., Kari, Z. A., Abdul Razab, M. K. A., Ahmed, H. A., Alagawany, M., & Gewaily, M. S. (2021). Selenium nanoparticles as a natural antioxidant and metabolic regulator in aquaculture: a review. Antioxidants, 10(9), 1364.
11. El-Beltagy, A., Elbakry, K., Elghazaly, M., Ali, L. S., & Daqaqq, N. (2019). Adverse effects of deltamethrin on the cerebellum of mothers rats and their offspring and the possible ameliorative role of melatonin. International Journal of Pure and Applied Zoology, 7(3).
12. El-Dien, H. M. S., El Gamal, D. A., Mubarak, H. A., & Saleh, S. M. (2010). Effect of fluoride on rat cerebellar cortex: light and electron microscopic studies. Egypt J Histol, 33, 245–246.
13. Elbanna, R., Osman, K. A., & Salama, M. S. (2023). Biomarkers of oral subacute toxicity of deltamethrin in exposed male Albino rats. Toxicology and Industrial Health, 39(12), 735–753.
14. Garman, R. H. (2011). Histology of the central nervous system. Toxicologic pathology, 39(1), 22–35.
15. Habib, G. M., Shi, Z.-Z., & Lieberman, M. W. (2007). Glutathione protects cells against arsenite-induced toxicity. Free Radical Biology and Medicine, 42(2), 191–201.
16. Hol, E. M., & Pekny, M. (2015). Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. Current opinion in cell biology, 32, 121–130.
17. Hussein, A. Y., EL-Shafey, R., & Elshazly, A. M. (2018). Ameliorative effect of pomegranate molasses on deltamethrin induced neurotoxicity in adult albino rats: Biochemical, histopathological & immunohistochemical study. The Egyptian Journal of Forensic Sciences and Applied Toxicology, 18(4), 83–102.
18. Kassab, A. A. (2018). Wheat germ oil attenuates deltamethrin-induced injury in rat cerebellar cortex: Histological and immunohistochemical study. Egyptian Journal of Histology, 41(2), 182–191.
19. Khalil, H. M., Azouz, R. A., Hozyen, H. F., Aljuaydi, S. H., AbuBakr, H. O., Emam, S. R., & Al-Mokaddem, A. K. (2022). Selenium nanoparticles impart robust neuroprotection against deltamethrin-induced neurotoxicity in male rats by reversing behavioral alterations, oxidative damage, apoptosis, and neuronal loss. Neurotoxicology, 91, 329–339.
20. Kim, L. H., Heck, D. H., & Sillitoe, R. V. (2024). Cerebellar functions beyond movement and learning. Annual review of neuroscience, 47.
21. Kumar, K., Patro, N., & Patro, I. (2013). Impaired structural and functional development of cerebellum following gestational exposure of deltamethrin in rats: role of reelin. Cellular and molecular neurobiology, 33(5), 731–746.
22. Li, J., Yu, Z., Han, B., Li, S., Lv, Y., Wang, X., Yang, Q., Wu, P., Liao, Y., & Qu, B. (2022). Activation of the GPX4/TLR4 signaling pathway participates in the alleviation of selenium yeast on deltamethrin-provoked cerebrum injury in quails. Molecular Neurobiology, 59(5), 2946–2961.
23. Lu, Q., Sun, Y., Ares, I., Anadón, A., Martínez, M., Martínez-Larrañaga, M.-R., Yuan, Z., Wang, X., & Martínez, M.-A. (2019). Deltamethrin toxicity: A review of oxidative stress and metabolism. Environmental research, 170, 260–281.
24. McElroy, C. L., Wang, B., Zhang, H., & Jin, K. (2024). Cerebellum and aging: update and challenges. Aging and Disease, 15(6), 2345.
25. McKimm, E., Corkill, B., Goldowitz, D., Albritton, L. M., Homayouni, R., Blaha, C. D., & Mittleman, G. (2014). Glutamate dysfunction associated with developmental cerebellar damage: relevance to autism spectrum disorders. The Cerebellum, 13(3), 346–353.
26. Meldrum, B. S. (2000). Glutamate as a neurotransmitter in the brain: review of physiology and pathology. The Journal of nutrition, 130(4), 1007S–1015S.
27. Moawad, R., Osman, A., Hassanein, K., Elkot, W., Asar, A., Alhag, S. K., Al-Shuraym, L. A., Alghamdi, O. A., Al-Farga, A., & Zaidalkilani, A. (2024). Assessment of sweet whey fortified with Bifidobacteria and selenium on reduction of pesticide liver toxicity in albino rats. Italian Journal of Food Science, 36(4), 275.
28. Mohamed, S. H. (2012). Vitamin E ameliorates histological and immunohistochemical changes in the cerebellar cortex of alloxan-induced diabetic rats. Egyptian Journal of Histology, 35(4), 650–659.
29. Ogaly, H. A., Khalaf, A., Ibrahim, M. A., Galal, M. K., & Abd-Elsalam, R. M. (2015). Influence of green tea extract on oxidative damage and apoptosis induced by deltamethrin in rat brain. Neurotoxicology and teratology, 50, 23–31.
30. Onaolapo, A., Onaolapo, O., & Nwoha, P. (2017). Methyl aspartylphenylalanine, the pons and cerebellum in mice: An evaluation of motor, morphological, biochemical, immunohistochemical and apoptotic effects. Journal of chemical neuroanatomy, 86, 67–77.
31. Patel, M. (2016). Targeting oxidative stress in central nervous system disorders. Trends in pharmacological sciences, 37(9), 768–778.
32. Pekny, M., & Pekna, M. (2014). Astrocyte reactivity and reactive astrogliosis: costs and benefits. Physiological reviews, 94(4), 1077–1098.

33. Petrovici, A., Savuța, G., Lucini, C., Robea, M.-A., & Solcan, C. (2025). Combined Neurotoxic Effects of Commercial Formulations of Pyrethroid (Deltamethrin) and Neonicotinoid (Imidacloprid) Pesticides on Adult Zebrafish (Danio rerio): Behavioral, Molecular, and Histopathological Analysis. Life, 15(4), 538.
34. Pitzer, E. M., Williams, M. T., & Vorhees, C. V. (2021). Effects of pyrethroids on brain development and behavior: Deltamethrin. Neurotoxicology and teratology, 87, 106983.
35. Rösen, P., Nawroth, P., King, G., Möller, W., Tritschler, H. J., & Packer, L. (2001). The role of oxidative stress in the onset and progression of diabetes and its complications: asummary of a Congress Series sponsored byUNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. Diabetes/metabolism research and reviews, 17(3), 189–212.
36. Salim, G., Rachid, R., & Mohamed, K. (2016). Deltamethrin induced neurodegeneration and behavioral effect by dysfunction cytosolic antioxidant system in rats brain. Algerian J. Nat. Sci, 1, 14–22.
37. Shehata, S. A., Kolieb, E., Ali, D. A., Maher, S. A., Korayem, H. E., Ibrahim, M. A., Nafie, M. S., & Ameen, S. H. (2024). Selenium alleviates modafinil-induced neurobehavioral toxicity in rat via PI3K/Akt/mTOR/GSK3B signaling pathway and suppression of oxidative stress and apoptosis: in vivo and in silico study. Environmental Science and Pollution Research, 31(1), 458–480.
38. Skalickova, S., Milosavljevic, V., Cihalova, K., Horky, P., Richtera, L., & Adam, V. (2017). Selenium nanoparticles as a nutritional supplement. Nutrition, 33, 83–90.
39. Surai, P. F. (2002). Natural antioxidants in avian nutrition and reproduction (Vol. 1). Nottingham University Press Nottingham.
40. Tedesco, A. M., Chiricozzi, F. R., Clausi, S., Lupo, M., Molinari, M., & Leggio, M. G. (2011). The cerebellar cognitive profile. Brain, 134(12), 3672–3686.
41. Turgut, M., Uysal, A., Pehlivan, M., Öktem, G., & Yurtseven, M. (2005). Assessment of effects of pinealectomy and exogenous melatonin administration on rat sciatic nerve suture repair: an electrophysiological, electron microscopic, and immunohistochemical study. Acta Neurochirurgica, 147(1), 67–77.
42. Wu, A., & Liu, Y. (2000). Apoptotic cell death in rat brain following deltamethrin treatment. Neuroscience letters, 279(2), 85–88.
43. Young, S. N. (2007). How to increase serotonin in the human brain without drugs. Journal of Psychiatry and neuroscience, 32(6), 394–399.
44. Zhang, S.-Q. (2023). Selenium and cognitive function. Metabolic Brain Disease, 38(1), 221–222.
45. Zhang, Z.-H., Peng, J.-Y., Chen, Y.-B., Wang, C., Chen, C., & Song, G.-L. (2023). Different effects and mechanisms of selenium compounds in improving pathology in Alzheimer's disease. Antioxidants, 12(3), 702.