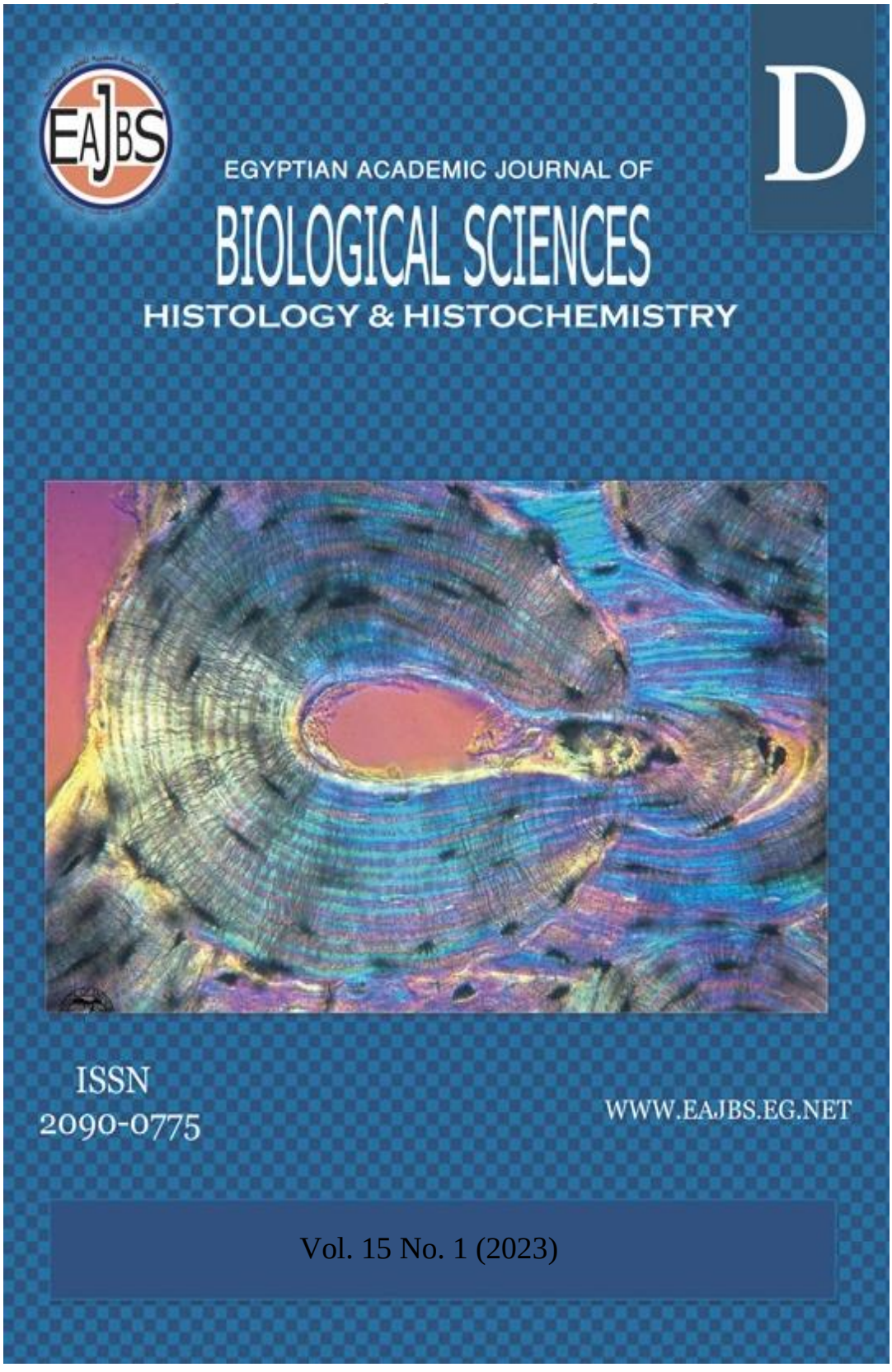
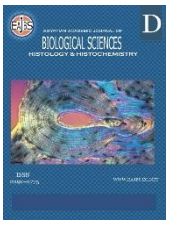


Citation: Egypt. *Acad. J. Biolog. Sci. (D-Histology and histochemistry) Vol.15(1) pp161-174(2023)*
DOI: 10.21608/EAJBSD.2023.298534

Egypt. Acad. J. Biolog. Sci., 15(1): 161-174 (2023)

Egyptian Academic Journal of Biological Sciences
D. Histology & Histochemistry
ISSN 2090 – 0775
http://eajbsd.journals.ekb.eg



Neurotoxic Effect of the Insecticide Fipronil on the Cerebellum of Rats and the Possible Protective Role of Vitamin E (Light and Immunohistochemical Study)

Abeer F. Abd El-Naeem and Zahraa M. Ismael
Human Anatomy and Embryology Department, Faculty of Medicine, Sohag University, Sohag
E.Mail* : abeerfareed167@gmail.com -zahraashour90@gmail.com.

ARTICLE INFO
Article History
Received:2/4/2023
Accepted:9/5/2023
Available:16/5/2023

***Keywords*:**
Fipronil- Vitamin E -Insecticide-caspase.

ABSTRACT

Background: Fipronil (FPN) is a widely indoor and agricultural used insecticide that is used by farmers all over the world. However, its incorrect use leads to the contamination of water and soil, so it became toxic to animals and humans. Vitamin E (VIT. E) is a fat-soluble vitamin in which its main role is to act as an antioxidant, removing free radicals that can destroy cells. So it is recommended for pregnant and lactating women, old age people and atherosclerotic patients. **Aim of the work:** studying the neurotoxic influence of Fipronil and the possible protective role of Vitamin E. **Material and methods:** this study was done on 30 adult rats divided equally into 3 groups: control group; with no intervention, 2nd group Fipronil; they were treated with Fipronil (20 mg/kg) daily orally for 5 days only, 3rd group (Fipronil + VIT.E); where the rats treated with VIT.E (1000 mg/kg) daily orally for 2weeks +Fipronil in the last 5days.the rats were anesthetized 24 hrs after the last dose and then sacrificed, and sections from the cerebellum were taken for the histological and immunohistochemical study. **Results**: Fipronil group showed destruction in the cells of the cortex with wide spaces in between. The positive reaction appears with caspase in this group, while less B-cell lymphoma-2 (Bcl-2) expression was noticed in this group, the 3rd group showed less destruction with Vitamin E intake. **Conclusion**: Fipronil is a toxic reagent and should be used with limitations; Vitamin E is a good antioxidant that should be used.

INTRODUCTION

Fipronil (5-amino-1-(2, 6-dichloro-α, α, α-trifluoro-p-tolyl) - trifluoromethyl sulfinyl pyrazole-3-carbonitrile) is a widespread insecticide which is a part of the family of phenylpyrazoles that acts well in the control of either indoor or agricultural pesticides (Tingle *et al.,2003*; Bonneau *et al.,2015*; Mossaa *et al.,2015*). It affects Gamma amino butyric acid (GABA) chloride receptors in insects which leads to neurogenic alteration and death (Gupta and Anadón,2018). In World Health Organization (WHO) it is classified as Class II toxicity (Saleh *et al.*,2020).

Their popularity is largely due to their easy application and their high toxicity to invertebrates, their spread to all organs needed, and their long persistence. However, they have the disadvantage of the probability of environmental contamination and exposure to other non-wanted organisms **(**Bonmatin *et al.*,2015). FPN is known to be less toxic in humans than insects, (Zhao *et al.*,2003) but it has a toxic effect on rats in doses of 40 to 100 mg/kg body weight according to US Environmental Protection Agency (2002).

Therefore, it may be more dangerous to humans than we expect (Rose *et al.,*1999). Oxidative stress and mitochondrial damage are the main causes of neurotoxicity induced by FPN (Seydi *et al.,*2021)**.** Neuronal cell death and even apoptosis arbitrated by FPN are due to the production toxic substances as reactive oxygen species (ROS) and also triggering of mitogen-activated protein kinase (MAPK) members (Ki *et al.,*2014). These ROS disturb the mitochondrial membrane permeability and so hinder the expression of some genes like (BCL-2) as an example, also it activates the Caspase cascade which leads to cellular apoptosis (Orrenius *et al.,*2007)**.** Vitamin E considered as one of the common lipid-soluble antioxidants, it exists in eight chemical forms that have multiple biological activity; α-tocopherol is the most active form of VIT. E: The antioxidant mechanism of action of it is by protecting the cell membranes from free radicals that generate from polyunsaturated fatty acids and by scavenging the superoxide and hydroxyl radicals (Lee and Ulatowski, 2019; Traber and Bruno,2020; Atkinson *et al.,* 2008).

The mechanism of entry of VIT.E into the central nervous system is due to VIT.E receptor class B type 1 (SRB1) levels (Goti *et al.,*2001)**.** It is noticed that VIT.E concentration in the membrane of the cerebellum and striatum was more than any other region in the brain so it affects especially their function (Mohn *et al.,*2017).In Alzheimer's Diseased Patients, long-term therapy with VIT.D and E either separately or both caused decreased morphological alteration of neurons and ameliorate learning and memory disorders, VIT. E is also said to prevent the memory of affection associated with post-traumatic stress disorder (PTSD) (Mehrabadi and Sadr,2020; Ahmed *et al.,*2020)**.** SO foods with high concentrations of VIT.E are recommended as Nuts, seeds, and vegetable oils, also green leafy vegetables have good amounts of it (U.S. Department of Agriculture, 2019).

Aim of the Work: evaluation of the neurotoxic effect of Fipronil and the possible protecting role of VIT.E

MATERIALS AND METHODS

Chemicals and Drugs:

Fipronil: with a trade name (cockroach killing bait) manufactured by jingda road jinxing industry park, china100162, applied from the local market of pesticides.

VIT.E: tablets (400mg), manufactured by Pharco Pharmacy, bought from a local pharmacy

Animals:

This study was done on thirty adult male albino rats aged 2-3 months and has a weight of 150–200 gm. They were all gained from the animal breeding house of Sohag University. Then were reared in cages under standardized temperature and light conditions according to the Institutional Animal Care and Use Committee (IACUC) at Sohag University with ethical approval number 5-12-2022-3. They were fed the normal rodent diet and water.

Experimental Design:

Acclimatization for 1 week before the experiment was done for rats, then rats were divided equally into 3 main groups: Group I: The Control group: which was 2ry divided into 2 equal groups each group had 5 rats. Subgroup IA: left with the normal diet and water without any intervention (negative control). Subgroup IB: received corn oil only once daily by oral gavage (positive control for both Fipronil and Vit E). Group II: received Fipronil daily in a dose of 20 mg/kg, 1/5 the lethal dose, once daily orally for 5 days only (Chagnon *et al.,*2015). Group III received 1ry Vit E at a dose of 1000 mg/kg once daily for 2 weeks then Fipronil added to them at the same dose of the previous group once daily orally at the last 5 days only (Selim *et al.,*2017). After 24 hrs from the last dose, the rats used were anesthetized, sacrificed, then dissected

and samples from the cerebellum were taken.

Histopathology and Immunohistochemistry:

Sample preparation for light microscopic study: 1st step was fixation in 10% neutral buffered formalin, then they were washed under running water, immersed in alcohol at serial dilutions then in paraffin, then slices of 5-7 µm thickness were cut and examined with a light microscope after staining with Hematoxylin and Eosin (H&E) (AlBasher *et al.*,2020).

For the immunohistochemical study with BCL-2, cerebellar sections were dehydrated with graded ethyl alcohol. Next, slide heating was done at 121 °C for 5 min in distilled water for antigen retrieval. At last, the slide was incubated with BCL-2(Banu *et al.*,2011).

For an immunohistochemical study with Caspase-3 which is used as an indicator for apoptosis, sections were bathed in phosphate-buffered saline for 5mins, then incubated with the anti-Caspase-3 antibody at 1:200 dilution at 4°C overnight then bathed with (1:500) secondary antibody for 1hr in room temperature. Samples after that were incubated in 3,3-diaminobenzidine for 10 min, counterstaining with Mayer's hematoxylin stain (TA060-MH) was done lastly. A positive reaction could be seen as brown discoloration.

Morphometric and Statistical Study:

The following measures were taken: thickness of the Purkinje layer, area percent of caspase and bcl-2 expression. For each section, 10 non-overlapping fields were taken. For the thickness of Purkinje layer we used sections of H and E magnification of 400, and for area percent of antigen expression we used sections with magnification 200. This was assisted by using Image J software (version 1.51k). The mean± SD (Mean ± standard deviation of the mean) was measured by SPSS program version 16. one-way analysis of variance (ANOVA) test was used, if it was statistically significant (P value ≤0.05) the post-hoc test would be used to discover the statistical difference among them (Stenberg *et al.*,2012).

RESULTS

Light Microscopic Results:

A. The positive and negative control gave no different results so they were all considered as control group.

The cerebellar cortex of control rats was seen formed of three layers; an outer molecular layer; a middle Purkinje cell layer; and an inner granular layer. The outer molecular layer appeared as a thick outer layer containing scattered nuclei of different sizes & shapes, either elongated, rounded, or satellites in shape**.** The Purkinje cell layer looked as a single row of nuclei in the deepest part of the molecular layer and just above the granular cell layer**,** Purkinje cells were large, with a piriform "flask" shape and relatively large nuclei. The granular cell layer was the deepest layer appeared, it was crowded with cells which were clumped in groups, with small rounded cell bodies (Figs. 1A and B).

B- Treated group II (fipronil): Molecular layer appeared less scattered with empty spaces in comparison with the control, Purkinje cell layer showed marked destruction with multiple empty spaces, some Purkinje cells were shrunken with pyknotic nuclei, the granular cell layer still be seen as groups of small sized darkly stained cells (Figs. 2A and B).

C- Treated group III (Fipronil + VIT.E):

Showed less destruction than group (II) in all different layers, the molecular layer changes were near the control group, the Purkinje layer showed less destruction with some pyknotic cells was founded, and the internal Granule layer was near the control group (Figs. 3 A and B).

With Immunohistochemistry:

(Caspase-3): Highest reaction (brown stain) was seen in the Fipronil group in comparison with with control group which showed a minimal reaction, and the VIT.E group which showed a less reaction of the antigen than the Fipronil group **(**Figs. 4 A, B and C).

(BCL-2): Less expression of the gene BCL-2 was seen in the Fipronil group compared with the control group which showed high expression of the gene, and the VIT.E group which showed more expression of the gene than Fipronil group (Figs. 5 A, B and C).

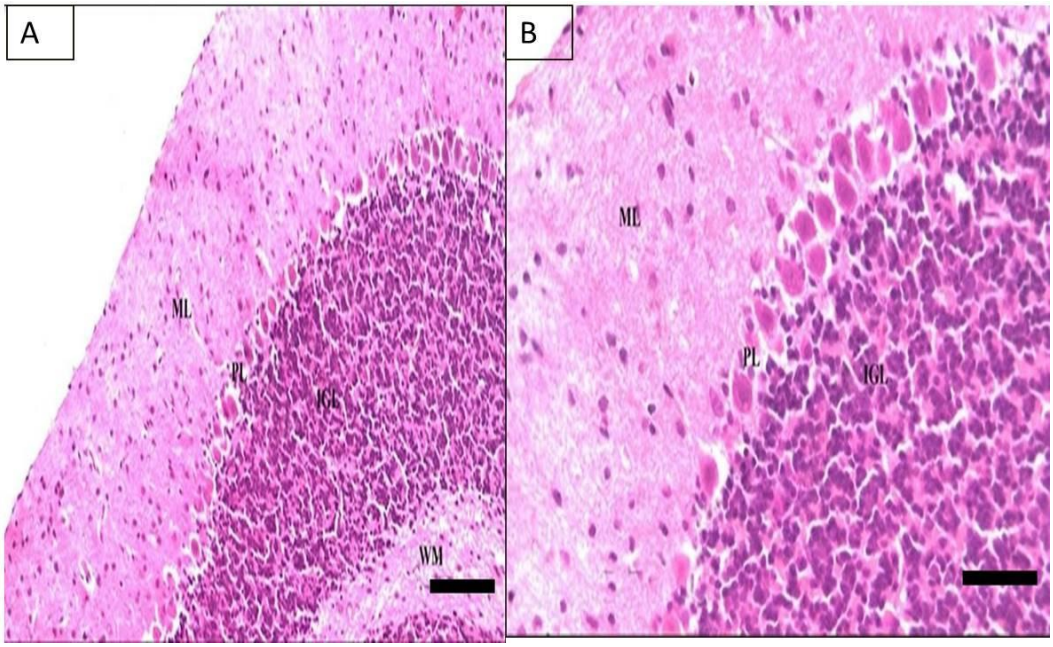


Fig. 1: Photomicrographs of a sagittal section of adult control cerebellum showing the three cortical layers, clear molecular layer **(ML),** Purkinje layer **(PL)** differentiated from other layers, and internal Granule layer **(IGL),** white matter **(WM) H&E(A X200), (B X400) scale bar =50um.**

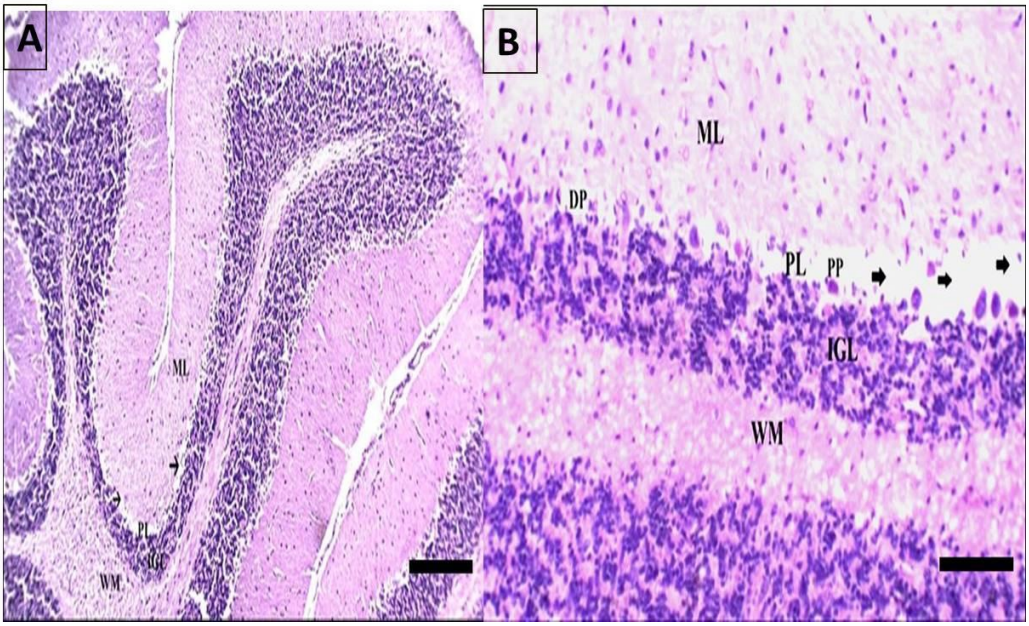


Fig. 2: Photomicrographs of a sagittal section of Fipronil treated cerebellum group (II): (**A)** less scattered cells appeared in the molecular layer **(ML),** Purkinje cell layer showed marked destruction with multiple empty spaces **(arrows),** internal granule layer **(IGL)** appeared crowded with cells, white matter **(WM) H&E (X200).** With high magnification (**B)** scattered cells appeared in the molecular layer **(ML),** Purkinje cell layer was markedly destructed **(arrows),** and multiple degenerated cells **(DP)** some were pyknotic **(PP),** internal granule layer **(IGL)** appeared crowded with cells **H&E (X400) scale bar =50um.**

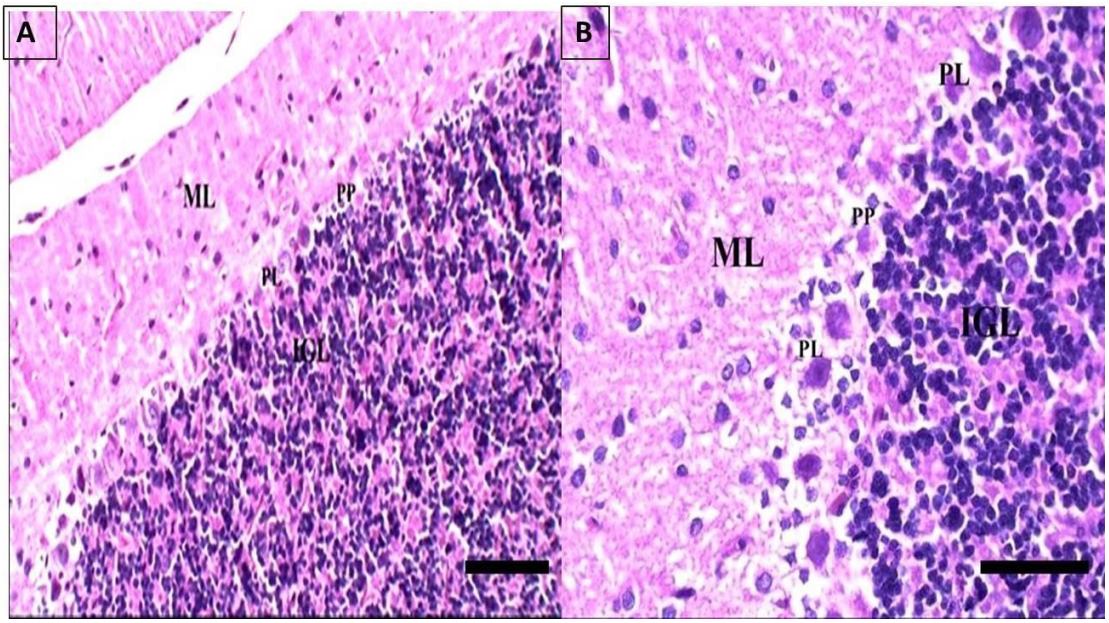


Fig. 3: Photomicrographs of a sagittal section of Fipronil and VIT.E treated cerebellum cortex of group (III)**: (A**) showed less destruction than group(II) in all different layers: the molecular layer **(ML)** appeared near the control group, Purkinje layer **(PL)** showed less destruction with some pyknotic cells **(PP)**, internal Granule layer **(IGL)** was the near control group. **H&E (X200).** With high magnification **(B**) showed near normal appearance in the molecular layer **(ML)**, Purkinje cell layer **(PL)** was less destructed than Fipronil treated group, some cells showed pyknosis **(PP)** and the internal granule layer **(IGL)** was near to the control group **H&E (X400) scale bar =50um.**

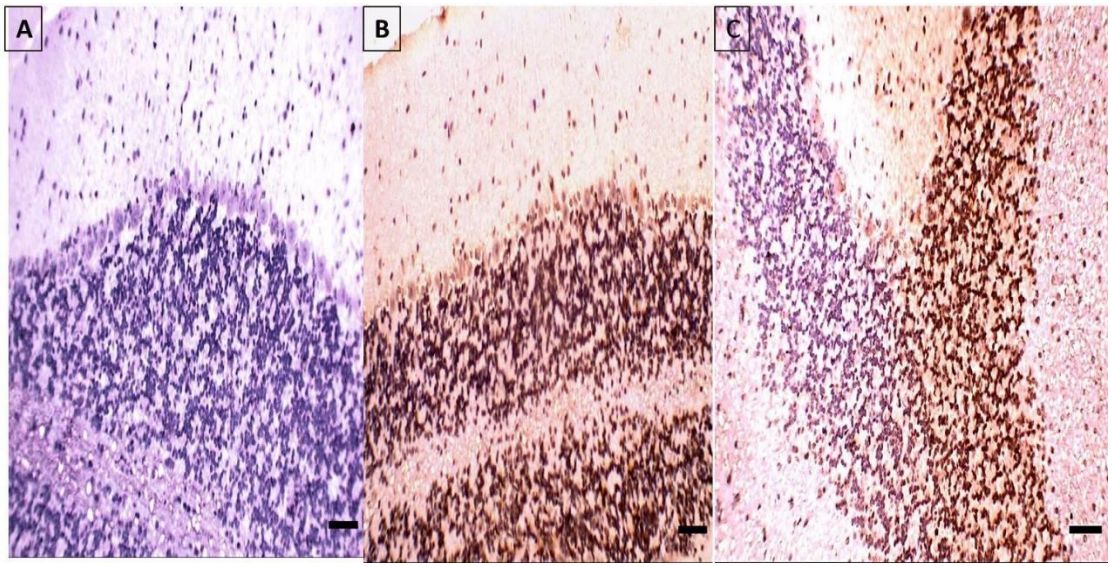


Fig. 4: Photomicrographs of the sagittal section of the cerebellum of the control rat with caspase-3 showed low expression of the antigen **(A)**. more expression of caspase-3 in the three layers; molecular layer **(ML),** Purkinje cell layer **(PL)** and internal Granule layer **(IGL)** in group (II) **(B)**. less expression to caspase-3 than Fipronil group **(PL)** in group (III) **(C) (X200) scale bar =50um.**

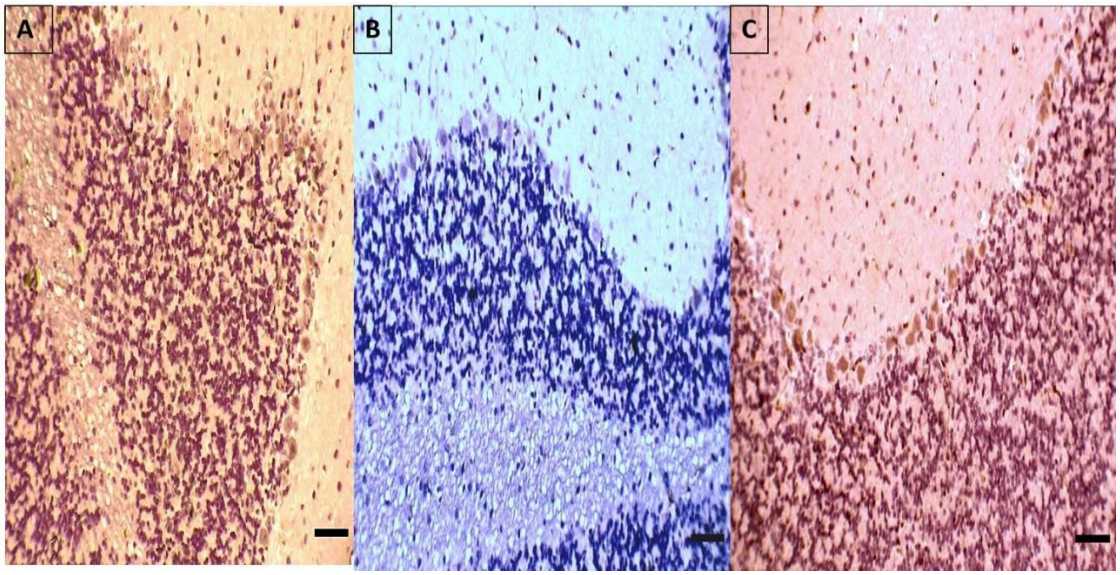


Fig. 5: Photomicrographs of sagittal section of the cerebellum of the control rat showed a week positive reaction to bcl-2**(A).** Fipronil group showed a negative reaction to bcl-2**(B)**. Fipronil and VIT.E group showed a positive reaction to bcl-2**(C) (X200) scale bar =50um.**

Statistical Results:

1. The mean thickness of the Purkinje layer in the control group was 87.4661±9.43775 pixels, while in group II (Fipronil) was 54.3331 ±7.67882 pixels, there was a highly significant decrease in group II (Fipronil) than the control group (p≤0.000), the thickness increased highly in group III (VIT.E) 65.0278 ± 11.71988pixel than group II (p≤.003), but decreased highly from the control (p≤0.000) (chart 1).

2. The mean area percent of caspase expression was 8.7840 ±.78507 in the control group, while in Fipronil group it was 18.7367±3.39804 with high significant increase than the control (p≤0.00). The mean area percent in Fipronil +VIT.E was 12.0902 ±.13545 with a highly significant decrease than group II (Fipronil) (p≤0.00), but also a highly significant increase than the control group p≤0.00 (chart 2).

3. The mean area percent of bcl-2 expression was 8.7624 ±.65449 in the control group, while in Fipronil group it was 4.0982±.69836 with highly significant decreases from the control (p≤0.00). The mean area percent in Fipronil +VIT.E was 7.5812± .52820with highly significant increase to group II Fipronil (p≤0.00), but also a significant decrease than the control group (p≤0.00) (chart 3).

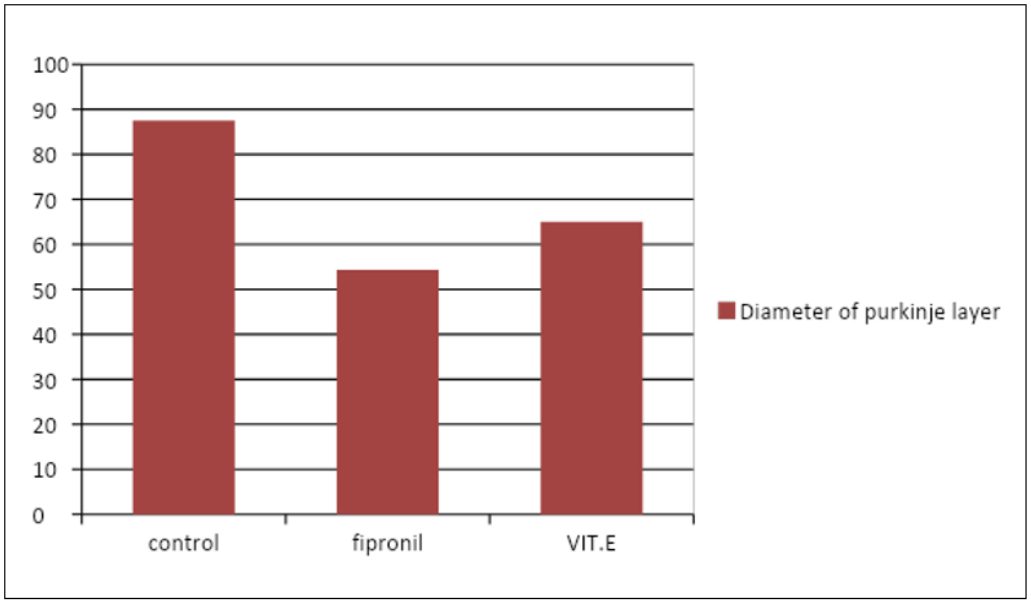


Chart 1: Diameter of Purkinje layer in different groups.

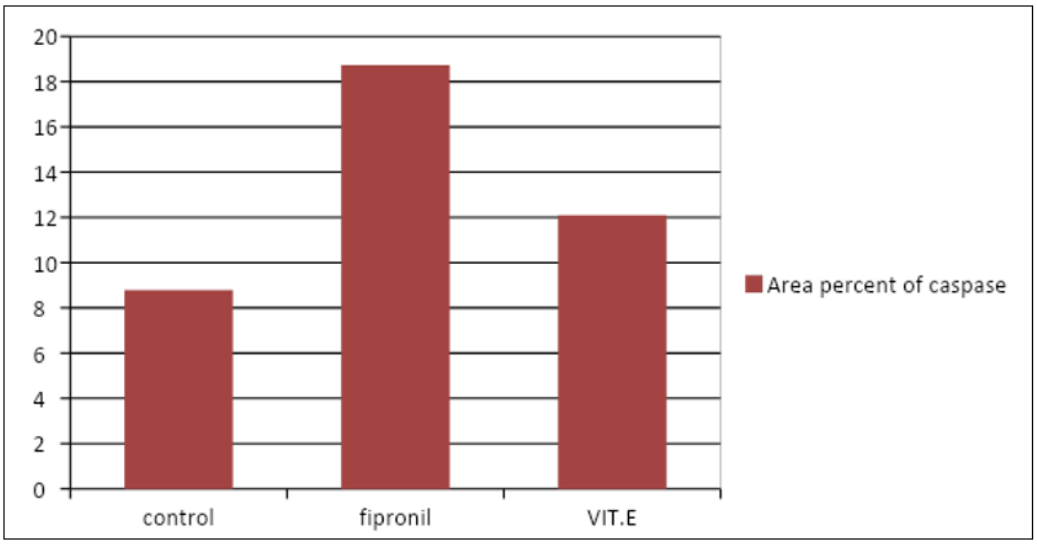


Chart 2: Area percent of caspase expression in different groups.

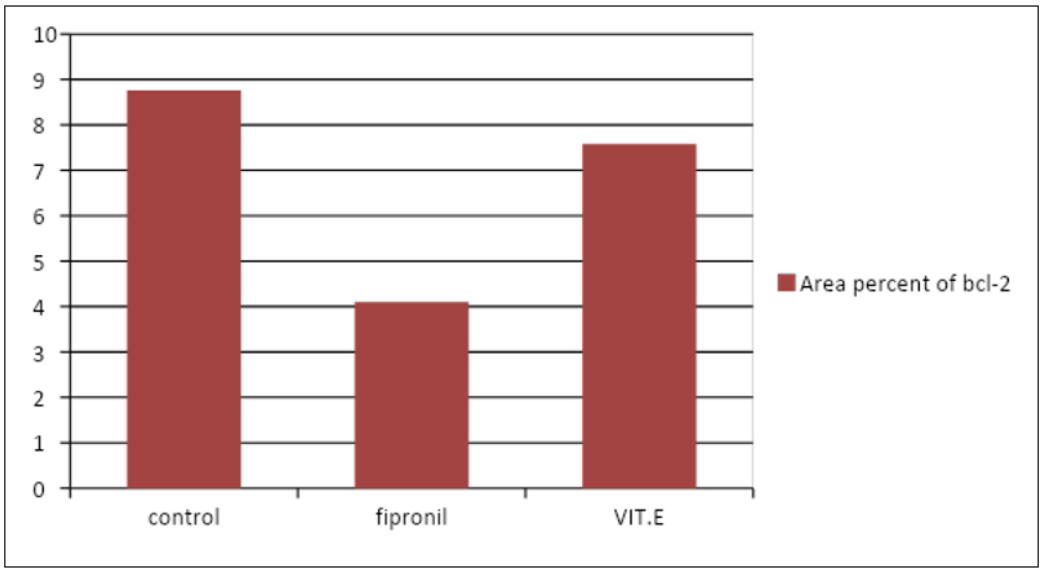


Chart 3: Area percent of bcl-2expression in different groups

DISCUSSION

Fipronil is a widespread pesticide in agriculture, domestic, and household environments because of its spread, its high toxicity, and easy application. However, it is highly toxic not only to target organisms but its toxicity is environmentally wide and other non-target organisms can be affected (Badgujar *et al.*, 2015; Das *et al.*,2006). The present study was done on the effect of FPN on cerebellar tissue and the possible protective effect of VIT.E on it by histological and immunohistochemical study. In this study rats treated with FPN expressed cell destruction and degeneration in cells which was accepted by Mahmoud *et al.* (2021) who studied the effect of FPN on the cerebral cortex and showed neurocytic degeneration with necrotic changes as represented by pyknotic and hyperchromatic nuclei. Also Elshony *et al.* (2021) said that the FPN group revealed shrunken Purkinje cells and loss of dendritic arborization. This may be due to oxidative stress which has been approved as one of the major causes of neuronal loss (Ogaly *et al.*,2015). FPN is considered one of the most neurotoxic insecticides used by farmers Al-Harbi, (2016) and Khalaf *et al.* (2019) showed also that FPN can cause nuclear pyknosis and degeneration in the neurons of the cerebral cortex, and the hippocampus.

Mahmoud *et al.* (2021) showed that exposure to FPN would increase brain redox balance. Redox balance is vital for maintaining cellular homeostasis (Ursini *et al.* 2016) and oxidative stress results from an imbalance between pro-oxidants and anti-oxidants (Conti *et al.* 2016). In our research; the rats treated with FPN showed more expression of caspase-3, which means more apoptosis of neuronal cells, this was accepted by (Khalaf *et al.*2019) who showed similar results to this
study.

Caspase is an enzyme that is activated in apoptosis after the release of cytochrome c caused by depolarization of the mitochondrial membrane due to oxidative stress (Wang and Youle,2009; Wang *et al.*2013) and exposure to Fipronil is the cause of apoptosis and increases oxidative stress as seen by Khan *et al.* (2015) reported more cell death by FPN. Vidau *et al.* (2011) said that more reduction in the mitochondrial membrane potential in exposure to FPN. Wang *et al.* (2016) reported also that FPN can cause intrinsic damage to brain tissue due to the generation of toxic factors such as reactive oxygen species (ROS) and/or reactive nitrogen species (RNS), which are the main causes of destruction. The BCL-2 protein family significantly controls mitochondrial integrity. Intrinsic apoptosis via mitochondrial damage causes reduced BCL-2 expression in the mitochondria as said by (Park *et al.*2016). Bcl-2 is an anti-apoptotic protein that regulates the apoptosis process (Liang *et al.*2001). It was reported that FPN decreased BCL-2 expression as mitochondrial membrane potential decreased with an increase in ROS generation after treatment with FIP (Zhang *et al.*2015; Lee and Koh, 2012) and it is reported that even a high dose of FIP (50 lM) did not increase Bcl-2 protein expression; which means that a high dose of FPN might cause cell damage through another route (Hu *et al.*2010)**.** In the present study adding VIT.E gave us near-normal results either on H and E stain, or immunohistochemical. VIT. E is a very important antioxidant that can prevent the damaging effects of many toxins and diseases (Sadeghi *et al.*2018)**.** As It is lipid-soluble so it is one of the main components of the lipid bilayer of the cell membrane, It also contains hydroxyl group that acts as a non-radical product and prevents oxidation by FPN (Niki *et al.*2014). These positive results were accepted by Sadeghi *et al.* (2019) who showed that vitamin E decreased signs of oxidative stress in the brain after exposure to Polyvinyl Chloride. Also, it

was approved that vitamin E administration in the developing cerebellum could reduce factors that induce cell apoptosis (Shirpoor *et al.*2009).

VIT.E increased Bcl-2 reaction while decreasing caspase expression, this means protection against apoptosis. This was accepted by Qin *et al.* (2006) who said that VIT.E increased the Bcl2 level in mitochondria, due to suppression of cytochrome C releases, and as a result caspase 9 and caspase 3 expression decreased. Also, Kandeila *et al.* (2018) who studied the effect of VIT.E and wheat on rat kidneys treated with gentamicin using bcl-2, found that bcl-2 levels increased in rats treated with VIT.E which means more survival and less apoptosis. The fate of cells whether to die or live is in direct relation to the balance between survival and apoptosis signaling (Banu *et al.*2011). VIT.E has a clear effect on glial cells against toxic effects of the chemical, metabolic, or toxic agents by protecting nerve tissues with clearance of free radicals and also protecting the cell membranes (Baydas *et al.*2003). Due to its antioxidant effect, VIT.E is advised in the prevention of numerous possible diseases such as cancer, arthritis and cataracts which are associated with reactive oxygen species molecules (Rizvi *et al.*2014).

CONCLUSION

Fipronil has a neurotoxic effect on rat cerebellum and should be used cautiously; Vitamin E has a protective effect on cerebellar tissue that should be taken for protection against pesticides' neurotoxic effects.

Limitations: Due to the limitation of the investigations done(limited on microscopic study only) , more research is needed to study the toxic effect of Fipronil on other organs, Vitamin E needs more research to evaluate its antioxidant effect against other toxic pesticides or pollutants.

Conflict Of Interest: No conflict of interest.

Authors' Contribution: ZA: Designed the study and forms data collection and data analysis, AA: Writing review, statistical analysis, revision.

REFERENCES

Ahmed, M., Alzoubi, K.H. & Khabour, O.F. (2020).Vitamin E prevents the cognitive impairments in post-traumatic stress disorder rat model: behavioral and molecular study. *Psychopharmacology (Berl).* 237(2):599-607. doi: 10.1007/ s00213-019-05395-w.

AlBasher, G., Abdel-Daim, M.M., Almeer, R., Ibrahim, K.A., Hamza, R.Z., Bungau, S. & Aleya, L. (2020). Synergistic antioxidant effects of resveratrol and curcumin against fipronil-triggered oxidative damage in male albino rats. *Environmental Science and Pollution Research.* 27(6): 6505-6514. doi: 10.1007/ s11356-019-07344-*8.*

Al-Harbi, MS. (2016). Fipronil induced hepatotoxicity, genotoxicity, oxidative stress and the possible ameliorative effect of Ginseng. *British Journal of Pharmaceutical Research*.: 14: 1–14.

Atkinson, J., Epand, R.F.& Epand, R.M. (2008). Tocopherols and tocotrienols in membranes: a critical review. *Free Radical Biology and Medicine.* 44(5): 739-64. doi: 10.1016/j. freeradbiomed.

Badgujar P.C, Pawar N.N, Chandratre G.A, Telang A.G, Sharma A.K. (2015). Fipronil induced oxidative stress in kidney and brain of mice: protective effect of vitamin E and vitamin C. *Pesticide Biochemistry and Physiology* (118):10-8. doi: 10.1016/j.pestbp.2014.10.013.

Banu, S.K., Stanley, J.A., Lee, J., Stephen, S.D., Arosh, J.A., Hoyer, P.B. & Burghardt, R.C. (2011). Hexavalent chromium-induced apoptosis of granulosa cells involves selective sub-

cellular translocation of Bcl-2 members, ERK1/2 and p53. *Toxicology and Applied Pharmacology*. 251(3):253-66. doi: 10.1016/j.taap.2011.01. 011.

Baydas, G., Nedzvetskii, V.S., Tuzcu, M., Yasar, A. & Kirichenko, S.V.(2003). Increase of glial fibrillary acidic protein and S-100B in hippocampus and cortex of diabetic rats: effects of vitamin E. *European Journal of Pharmacology*.462(1-3):67-71. doi: 10.1016/s0014-2999(03) 01294-9.

Bonmatin, J.M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E.A., Noome, D.A., Simon-Delso, N. & Tapparo, A. (2015). Environmental fate and exposure; neonicotinoids and fipronil. *Environmental Science and Pollution Research*. 22(1):35-67. doi: 10.1007/ s11356-014-3332-7.

Bonneau, S., Reymond, N., Gupta, S. & Navarro C. (2015). Efficacy of a fixed combination of permethrin 54.5% and fipronil 6.1% (Effitix) in dogs experimentally infested with *Ixodes ricinus*. *Parasites Vectors*, 204 (8). https://doi. org /10.1186/s13071-015-0805-6

Chagnon, M., Kreutzweiser, D., Mitchell, E.A., Morrissey, C.A., Noome, D.A. & Van der Sluijs, J.P. (2015). Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environmental Science and Pollution Research* .22(1):119-34. doi: 10.1007/ s11356-014-3277-x.

Conti, V., Izzo, V., Corbi, G., Russomanno, G., Manzo, V., De Lise, F., Di Donato, A. & Filippelli, A. (2016). Antioxidant Supplementation in the Treatment of Aging-Associated Diseases. *Frontiers in Pharmacology*. 12(7):24. doi: 10.3389/fphar.2016.00024.

Das, P.C., Cao, Y., Cherrington, N., Hodgson, E. & Rose, R.L. (2006). Fipronil induces CYP isoforms and cytotoxicity in human hepatocytes. *Frontiers in Pharmacology*, 164(3):200-14. doi: 10.1016/j.cbi.2006. 09. 013.

Elshony, N., Nassar, A.M.K., El-Sayed, Y.S., Samak, D., Noreldin, A., Wasef, L., Saleh, H., Elewa, Y.H.A., Tawfeek, S.E., Saati, A.A., Batiha, G.E., Tomczyk, M., Umezawa, M.& Shaheen, H.M. (2021). Ameliorative Role of Cerium Oxide Nanoparticles Against Fipronil Impact on Brain Function, Oxidative Stress, and Apoptotic Cascades in Albino RatsFrontiers in Neuroscience,15:651471. doi: 10.3389/fnins.2021.651471.

Goti, D., Hrzenjak, A., Levak-Frank, S., Frank, S., van der Westhuyzen, D.R., Malle, E. & Sattler, W. (2001). Scavenger receptor class B, type I is expressed in porcine brain capillary endothelial cells and contributes to selective uptake of HDL-associated vitamin E. *Journal of Neurochemistry*, 76(2):498-508. doi: 10.1046/j.1471-4159. 2001.00100. x.

Gupta, R.C, and Anadón A. (2018). Fipronil, In Veterinary *Toxicology*, 3rd ed.; Gupta, R.C., Ed.; *Academic Press: Cambridge, MA, USA*, 42:533–538.

Hu, X.F., Li, J., Zhuang, W.& Li, L. (2010). Effect of fipronil on apoptosis rate and Bcl-2 of PC12 cells. *Chinese journal of industrial hygiene and occupational diseases*, 28(4): 264-7. Chinese. PMID: 20465951.

Kandeila M, Hassaninb K, Mohammeda E, Safwata G and Mohamed D.

(2018). Wheat germ and vitamin E decrease BAX/BCL-2 ratio in rat kidney treated with gentamicin. *Beni-Suef University Journal of Basic and Applied Sciences,* 7 (3): 257-262. https://doi.org/10.1016/ j. bjbas.2018.02.001.

Khalaf, A.A., Galal, M.K., Ibrahim, M.A., Allah, A.A.A., Afify, M.M. & Refaat, R. (2019). The *Terminalia laxiflora* modulates the neurotoxicity induced by fipronil in male albino rats. *Bioscience Reports*. 39(3): BSR20181363. doi: 10.1042/ BSR20181363.

Khan, S., Jan, M.H., Kumar, D. & Telang, A.G. (2015). Firpronil induced spermotoxicity is associated with oxidative stress, DNA damage and apoptosis in male rats. *Pesticide Biochemistry and Physiology*, (124):8-14. doi: 10.1016/j. pestbp.2015.03.010.

Ki, Y.W., Lee, J.E., Park, J.H., Shin, I.C.& Koh, H.C. (2012). Reactive oxygen species and mitogen-activated protein kinase induce apoptotic death of SH-SY5Y cells in response to fipronil. *Toxicology Letters*, 211(1):18-28. doi: 10.1016/j. toxlet.2012.02.022.

Lee YJE and Koh JHC. (2012). Reactive oxygen species and mitogen-activated protein kinase induce apoptotic death of SH-SY5Y cells in response to fipronil. *Toxicology Letters,* 1(211): 18-28.

Lee, P. & Ulatowski, L.M. (2019).Vitamin E: Mechanism of transport and regulation in the CNS. *IUBMB Life*, 71(4): 424-429. doi: 10.1002/iub. 1993.

Liang, Y., Yan, C. & Schor, N.F. (2001). Apoptosis in the absence of caspase 3, *Oncogene*, 20(45):6570-8. doi: 10.1038/sj. onc.1204815.

Mahmoud, Y.K., Ali, A.A., Abdelrazek, H.M.A., Aldayel, T.S., Abdel-Daim, M.M. & El-Menyawy, M.A.I. (2021). Neurotoxic Effect of Fipronil in Male Wistar Rats: Ameliorative Effect of L-Arginine and L-Carnitine. *Biology (Basel),*10 (7):682. doi: 10.3390/biology 10070682.

Mehrabadi, S. & Sadr, S.S. (2020). Administration of Vitamin D_3 and E supplements reduces neuronal loss and oxidative stress in a model of rats with Alzheimer's disease. *Neurological Research and Practice. 42(10):862-868. doi: 10.1080/01616412.2020.1787624.*

Mohn, E.S., Kuchan, M.J., Erdman, J.W., Neuringer, M., Matthan, N.R., Chen, C.O. & Johnson, E.J. (2017). The Subcellular Distribution of Alpha-Tocopherol in the Adult Primate Brain and Its Relationship with Membrane Arachidonic Acid and Its Oxidation Products. *Antioxidants (Basel),* .6(4):97. doi: 10.3390/antiox6040097.

Mossa, A.H., Swelam, E.S. & Mohafrash, S.M.M. (2015). Sub-chronic exposure to fipronil induced oxidative stress, biochemical and histopathological changes in the liver and kidney of male albino rats. *Toxicology Reports*. 2(19):775-784. doi: 10.1016/j. toxrep.2015.02.009.

Niki, E. (2014). Role of vitamin E as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence. *Free Radical Biology and Medicine,* (66):3-12. doi: 10.1016/j.freeradbiomed.

Ogaly, H.A., Khalaf, A.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. doi: 10.1016/j.ntt.2015.05.005.

Orrenius, S., Gogvadze, V. & Zhivotovsky, B. (2007). Mitochondrial oxidative stress: implications for cell death. *Annual Review of Pharmacology and Toxicology*, 47:143-83. doi: 10.1146/ annurev.pharmtox.

Park, J.H., Park, Y.S., Lee, J.B., Park, K.H., Paik, M.K., Jeong, M.& Koh, H.C. (2016). Meloxicam inhibits fipronil-induced apoptosis via modulation of the oxidative stress and inflammatory response in SH-SY5Y cells. *Journal of Applied Toxicology*, 36(1):10-23. doi: 10.1002/jat.3136.

Qin, F., Yan, C., Patel, R., Liu, W. & Dong, E. (2006). Vitamins C and E attenuate apoptosis, beta-adrenergic receptor desensitization, and sarcoplasmic reticular Ca2+ ATPase downregulation after myocardial infarction. *Free Radical Biology and Medicine,*40(10):1827-42. doi: 10.1016/j.freeradbiomed.

Rizvi, S., Raza, S.T., Ahmed, F., Ahmad, A., Abbas, S. & Mahdi, F. (2014). The role of vitamin e in human health and some diseases. *Sultan Qaboos University Medical Journal*, 14(2): e157-65. PMC3997530.

Rose RL, Hodgson E and Roe RM. (1999). Pesticides. In Marquardt H., Schafer S.G., McClellan R., Welsch F., editors. *Toxicology. Academic; San Diego, CA*p: 663–697.

Sadeghi A and Ghahari L. (2018). Vitamin E as an antioxidant reduces apoptosis of cells in rat hippocampus following exposure to polyvinyl chloride. *Annals of Military and Health Sciences Research.* 16(4): e85275. doi: 10.5812/amh. 85275.

Sadeghi A, Ghahari L and Yousefpour M. (2019). Vitamin E Supplementation Reduces Oxidative Stress in the Male Wistar Rats' Brain Against Polyvinyl Chloride Products. *Annals of Military and Health Sciences Research*, 17(2). doi: 10.5812/amh.92768.

Saleh, H., Nassar, A.M.K., Noreldin, A.E., Samak, D., Elshony, N., Wasef, L., Elewa, Y.H.A., Hassan, S.M.A., Saati, A.A., Hetta, H.F., Batiha G.E., Umezawa, M., Shaheen, H.M.& El-Sayed, Y.S. (2020). Chemo-Protective Potential of Cerium Oxide Nanoparticles against Fipronil-Induced Oxidative Stress, Apoptosis, Inflammation and Reproductive Dysfunction in Male White Albino Rats. *Molecules*, 25(15):3479. doi: 10.3390/molecules25153479.

Selim, A., Khalaf, M.M., Gad, A.M. & Abd El-Raouf, O.M. (2017). Evaluation of the possible nephroprotective effects of vitamin E and rosuvastatin in amikacin-induced renal injury in rats. *The Journal of Biochemical and Molecular Toxicology,*31(11). doi: 10.1002 /jbt.21957.

Seydi, E., Mehrpouya, L., Sadeghi, H., Rahimi, S. & Pourahmad, J. (2021). Luteolin attenuates Fipronil-induced neurotoxicity through reduction of the ROS-mediated oxidative stress in rat brain mitochondria. *Pesticide Biochemistry and Physiology*, 173:104785. doi: 10.1016/j. pestbp.

Shirpoor, A., Salami, S., Khadem-Ansari, M.H., Minassian, S., Yegiazarian, M. (2009). Protective effect of vitamin E against ethanol-induced hyperhomocysteinemia, DNA damage, and atrophy in the

developing male rat brain. *Alcohol: Clinical and Experimental Research,* 33(7): 1181-6. doi: 10.1111/j.1530-0277.

Stenberg, L., Kanje, M., Dolezal, K. & Dahlin, L.B. (2012). Expression of activating transcription factor 3 (ATF 3) and Caspase 3 in Schwann cells and axonal outgrowth after sciatic nerve repair in diabetic BB rats. *Neuroscience Letters,* 515(1): 34-8. doi: 10.1016/j.neulet.2012 .03.011.

Tingle, C.C.D., Rother, J.A., Dewhurst, C.F., Lauer, S. & King, W.J. (2003). Fipronil: Environmental Fate, Ecotoxicology, and Human Health Concerns. In: Ware, G.W. (eds) Reviews of Environmental Contamination and Toxicology. *Reviews of Environmental Contamination and Toxicology,* (176)1-66. Springer, New York, NY. https://doi.org/10.1007/978-1-4899-7283-5_1.

Traber MG and Bruno RS. (2020). Vitamin E. In BP Marriott, DF Birt, VA Stallings, AA Yates editors. Present Knowledge in Nutrition, Eleventh Edition. London, United Kingdom: *Academic Press (Elsevier)*. (2020); 115–36.

U.S. Department of Agriculture, Agricultural Research Service, Food Data Central. (2019). https://fdc.nal.usda.gov/download-datasets.html

Ursini, F., Maiorino, M. & Forman, H.J. (2016). Redox homeostasis: The Golden Mean of healthy living. *Redox Biology,* (8):205-15. doi: 10.1016/j.redox.2016. 01.010.

US Environmental Protection Agency. EPA 738-R-01-007; *Washington*, DC: (2002). Interim Reregistration Eligibility Decision for Chlorpyrifos. Prevention, Pesticides and Toxic Substances. Office Of Prevention, Pesticides and Toxic Substances.

Vidau, C., González-Polo, R.A., Niso-Santano, M., Gómez-Sánchez, R., Bravo-San Pedro, J.M., Pizarro-Estrella, E., Blasco, R., Brunet, J.L., Belzunces, L.P.& Fuentes, J.M. (2011). Fipronil is a powerful uncoupler of oxidative phosphorylation that triggers apoptosis in human neuronal cell line SHSY5Y. *Neurotoxicology,* 32(6):935-43. *doi: 10.1016/j.neuro.2011. 04. 006.*

Wang XQ, Li YG, Zhong S, Zhang H, Wang XY, Qi PP and Xua H. (2012). Oxidative injury is involved in fipronil-induced G2/M phase arrest and apoptosis in Spodoptera frugiperda (Sf9) cell line. *Pesticide Biochemistry and Physiology,* 2013; 105: 122–130. http://dx.doi.org/10. 1016 /j.pestbp...12.008.

Wang, C. & Youle, R.J. (2009). The role of mitochondria in apoptosis. *The Annual Review of Genetics*, (43):95-118. doi: 10.1146/ annurev-genet-102108-134850.

Wang, X., Martínez, M.A., Wu, Q., Ares, I., Martínez-Larrañaga, M.R., Anadón, A. & Yuan, Z. (2016). Fipronil insecticide toxicology: oxidative stress and metabolism .*Critical Reviews in Toxicology*, 46(10):876-899. doi: 10.1080/ 10408444.2016.1223014.

Zhang, B., Xu, Z., Zhang, Y., Shao, X., Xu, X., Cheng, J. & Li, Z. (2015). Fipronil induces apoptosis through caspase-dependent mitochondrial pathways in Drosophila S2 cells. *Pesticide Biochemistry and Physiology,* (119):81-9. doi: 10.1016/j.pestbp.2015. 01. 019.

Zhao, X., Salgado, V.L., Yeh, J.Z. & Narahashi, T. (2003).

Differential actions of fipronil and dieldrin insecticides on GABA-gated chloride channels in cockroach neurons. *The Journal of Pharmacology and Experimental Therapeutics*. 306 (3):914-24. doi: 10.1124/ jpet. 103.051839.

ARABIC SUMMARY

التسمم العصبى الناشئ عن المبيد الحشرى فيبرونيل على المخيخ الخاص بالجرذان والتأثير الوقائي المحتمل لفيتامين ه.دراسة هستولوجية وهستولوجية مناعية

عبير فريد عبدالنعيم ،و زهراء محمد اسماعيل
قسم التشريح الأدمى وعلم الاجنة – كلية الطب- جامعة سوهاج

المقدمة : يعتبر فيبرونيل من المبيدات الحشرية واسعة الانتشار سواء داخل المنازل أو فى الزراعة وهو يستخدم عن طريق المزارعين على مستوى العلم كله.ومع ذلك الاستخدام الخاطئ له أو التعرض المفاجئ له قد يؤدى الى تلوث الماء والتربة ويصبح بذلك سام للانسان والحيوان.أيضا التعرض له لفترة طويلة قد يسبب تسمم عصبى ,تسمم كبدى أو كلوى.فى المقابل يعد فيتامين ه من الفيتامينات التى تذوب فى الدهون والتى هى من المضادات القوية للأكسدة ,حيث تزيل المواد المؤكسدة التى تدمر الخلية.بجانب انها تدعم الجهاز المناعى وتمنع التجلطات من التكون.ولذلك فانها ينصح بها للذكور والاناث بعد عمر 14 عام,وينصح ايضا بها للحوامل والمرضعات.
هدف البحث: أجرى البحث لدراسة التسمم العصبى الناشئ عن مادة فيبرونيل والتأثير الوقائى المحتمل لفيتامين ه.
المواد المستخدمة:أجريت الدراسة على عدد 30 جرذ حيث تم تقسيمهم الى 3 مجموعات متساوية:المجموعة الضابطة:لم تتناول أى مواد,المجموعة الثانية (فيبرونيل):حيث تم اعطاء الجرذان مادة فيبرونيل بجرعة 20مجم لكل كجم يوميا عن طريق الفم لمدة 5 أيام فقط,الجموعة الثالثة:تم اعطائها فيتامين ه يوميا عن طريق الفم بجرعة 1000مجم لكل كجم +مادة فيبرونيل اخر 5 ايام فقط بنفس الجرعة السابقة.بعد مرور 24 ساعة من اخر جرعة تم تخدير الفئران والتضحية بهم ,تم اخذ عينات من المخيخ لدراستها هستولوجيا ومناعيا.
النتائج:الجرذان التى تم اعطائها مادة فيبرونيل فقط أظهرت تدمر فى الخلية على مستوى طبقات القشرة وأظهرت مسافات واسعة بينهم.ظهر تفاعل ايجابى أكثر مناعيا مع هذه المجموعة لمادة كاسبز-3.وأظهرت أقل تفاعل مناعى لمادة ب س ل-2.المجموعة الثالثة أظهرت أقل دمار للخلية مع فيتامين ه.

الخلاصة: مادة فيبرونيل هى مادة مدمرة يجب الحد من استخدامها ,بينما فيتامين ه هو مضاد للاكسدة قوى يجب استخدامه.