Journal of Applied and Natural Science
14(1), 120 - 126 (2022)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Abamectin exposure during lactation triggering oxidative stress and expression pattern of Bcl-2 perturbation in rat pups brain

Reem M Ziada
Mammalian and Aquatic Toxicology Department, Central of Agricultural Pesticides Laboratory, Agricultural Research Center, Egypt

Email: zorosonds@yahoo.com

Article Info
https://doi.org/10.31018/jans.v14i1.3227
Received: December 21, 2021
Revised: February 13, 2022
Accepted: February 17, 2022

How to Cite
Ziada, R. M. (2022). Abamectin exposure during lactation triggering oxidative stress and expression pattern of Bcl-2 perturbation in rat pups brain. *Journal of Applied and Natural Science*, 14(1), 120 - 126. https://doi.org/10.31018/jans.v14i1.3227

Abstract
Pesticides, for example, are thought to be essential drivers of brain injury and dysfunction. Abamectin (ABA) is by far the most routinely used pesticide in farming and healthcare, and its toxicity to nontarget organisms has attracted considerable interest. The effect of abamectin pesticide delivered at the postnatal period on the antioxidant defense system was assessed. This study also examined apoptotic gene expression indicators in the brains of rat pups over neonatal weeks. Pregnant Wister rats were split into two groups: G1 received deionized water (control), and G2 received 0.211 mg/L of abamectin. The first day of abamectin exposure was the first day after delivery and continued until the tenth day of lactation. After 10 days (mid lactation) and the lactation period, rat pup brain samples were taken for oxidative biochemistry investigations and apoptotic gene expression (RT–qPCR). In comparison to the control group G1, Abamectin reinforces protein carbonyl levels and glutathione-based enzymes (transferase and peroxidase), whereas superoxide dismutase and glutathione levels are reduced in the pup's brain. Furthermore, Abamectin induced considerable upregulation of proapoptotic (Bax) and antiapoptotic (Bcl-2) mRNA gene expression. Overall, our findings characterize the relationship between brain changes and abamectin administration during lactation periods, even at low doses that are considered safe, and indicate that this abamectin insecticide is harmful to the growing neurological system.

Keywords: Abamectin; Apoptosis; Brain, Lactation, Oxidative stress

INTRODUCTION

Pesticides are a genuine issue for health, particularly the brain, especially when they are available at excessive levels during critical stages of brain development because of their extensive use in agriculture and the high probability of finding them in food and water (Jurewicz and Hanke, 2008). Abamectin is by far the most routinely used pesticide in farming and healthcare, and its toxicity to nontarget organisms has attracted considerable interest (Bai and Ogbourne, 2016). Excessive application of abamectin leads to an increase in its residues in crops and persistence in water, soil, sediment, and food products (Danaher *et al.,* 2012). Abamectin is a neurotoxin that affects glutamate amino butyric acid-gated chloride channels in brain cells. Because the blood–brain barrier guards neurons in vertebrates, it is considered mainly safe for vertebrate animals (Omura, 2008). The persistence of abamectin in the environment poses a threat to ecosystems in various habitats. Macrocyclic lactones may cross the blood–brain barrier, causing GABA-like toxic effects such as hyperexcitability, incoordination, tremors, and hypotension, which then progresses to ataxia, coma, respiratory failure, and even deafness (Yang, 2012). Exposure to abamectin residues over an extended period through some foods, such as crops, may promote premature aging of stomach cells due to excessive ROS accumulation, as well as degenerative disorders, including gastric ulcers and even gastric cancer (Zhu *et al.,* 2019), where oxidative stress is a major component of avermectin-induced cytotoxicity (Zhu *et al.,* 2013)

Pesticides, for example, are known to harm neuronal cytoarchitecture and are therefore regarded as essential predictors of brain dysfunction (N'Go *et al.,* 2013). It is also worth noting that early disruptions in brain development might result in neurologic issues either throughout infancy or at a late stage in maturity (Olney *et al.,* 2002). In keeping with this, early postnatal pesti-

cide exposure has been demonstrated to influence brain development, and a growing body of research suggests that early pesticide exposure has neurobehavioral implications (Heyer and Meredith, 2017). Furthermore, an epidemiological survey found a direct association between the increased use of agricultural chemicals and the prevalence of multiple neurological illnesses at various ages, including autism, dementia, and anxiety disorder (Seneff and Li, 2015). Biochemical markers that might express energy distribution characteristics or be linked to other individual endpoints, such as breeding or development and population health viability, can assist investigators in better understanding the processes associated with environmental stressor exposure (Sokolova *et al.,* 2012). Neuronal proliferation, mass migration, differentiation, synaptogenesis, gliogenesis, myelination, and programmed cell death are required for optimal central nervous system development (Mattson, 2006). Whereas the redox system plays a vital role in cell viability and mortality, oxidative stress is caused by the accumulation of oxidative damage products that can lead to a dysregulation of apoptosis and autophagy, leading to diseases including aging, degenerative disorders, and cancer. Apoptosis, or programmed cell death, is a natural suicide mechanism that occurs during development and as a homeostatic mechanism to keep cell populations in tissues in check (Muñoz-Pinedo, 2012). Neuronal loss is part of a remodelling process that eliminates approximately half of all neurons born during neurogenesis. Following this developmental window, neuronal loss is physiologically inappropriate for most systems and can contribute to neurological impairments such as Alzheimer's and Parkinson's disease. Indeed, the central nervous system is highly vulnerable to the detrimental effects of chemical or physical stimuli during these two critical times (O'Rahillyand Müller, 2008). Targeted disruption of the Bcl-2 family results in neurodevelopmental abnormalities that primarily affect the maintenance of select neuronal subpopulations during postnatal life. Bcl-2 family members may play a more significant role in the embryonic brain (González-García *et al.,* 1994). Bax, a member of the Bcl-2 family, plays an important role in releasing apoptogenic factors from mitochondria (Liou *et al.,* 2014; Youle and Strasser, 2008).

Therefore, this study aimed to investigate the relationship between abamectin exposure during critical periods of life (lactation) and the developing brain in rat neonates by determining oxidative stress biomarkers and the expression pattern of genetic apoptosis biomarkers.

MATERIALS AND METHODS

Pesticides used

Abamectin formulation (1.8% emulsifiable concentrate, EC) was obtained from Mammalian Toxicology Department, Central Agricultural Pesticides Lab., Agriculture Research Center, Dokki, Giza, Egypt.

Animals and experimental design

The test was carried out following the guidelines for the care and use of laboratory animals (Council, 2011). Twenty timed-pregnant rats were withdrawn from the breeding colony of the Mammalian Toxicology Department, Central Agricultural of Pesticides Laboratory., and Agriculture Research Center. Animals were given a well-balanced diet and unlimited access to tap water. The animals were maintained in separate cages in an air-conditioned room at a temperature of 32 degrees Fahrenheit. 23±2 °C and a relative humidity of ~ 55% (50 - 60%) under a normal light/dark cycle. Immediately after delivery (postnatal day zero), the pups were weighed, counted, sexed, and checked for anomalies and then breasted feeding for each corresponding dam. Lactating dams and their pups were assembled into two main experimental groups. The first one, G1 (10 dams), was saved as the control group and received distilled water daily throughout the lactation period. The second group, G2 (10 dams), was incubated with 0.211 mg/kg abamectin from the first day of lactation until the 10^{th} day.

After 10 and 21 days, the neonatal rats (everyone from the different dams to avoid the siblings) were sacrificed without anesthesia. The brain was obtained, splashed with ice-cooled normal saline solution, and quickly frozen until biochemical and gene expression assays were used.

Biochemical markers

Tissue preparation

The brain tissues were homogenized (1:10% W/V) in ice-cold sodium phosphate buffer (50 mM, pH 7.4) containing 0.1 mM ethylenediaminetetraacetic acid. The homogenates were centrifuged at 12.000 g for 30 min at 4 °C, and the supernatant was directly frozen until use. The total protein content was determined based on the method of Bradford (1976).

Estimation of oxidative stress biomarkers

Protein carbonyl content was spectrophotometrically determined according to the method of (Reznick and Packer, 1994), while reduced glutathione (GSH) was determined by the method of (Ellman, 1959). The antioxidant enzymes Gp_x, SOD, and GST were determined by the methods of Necheles *et al.* (1969), Marklund and Marklund (1974), and Habig *et al.* (1974), respectively.

Apoptotic gene expression estimation

A QIAamp RNeasy Mini Kit (Qiagen, Germany, GmbH, Catalogue no.74104) was used to extract RNA from the brain. According to the manufacturer's instructions,

matching cDNA was generated using RevertAid Reverse Transcriptase Thermo Fisher (catalogue number: K1622). Real-time polymerase chain reaction (RT–PCR) quantification was performed by a Stratagene MX3005P instrument using the Quantitect SYBR green PCR kit (Cat. No. 204141) with a 25 µl total reaction volume containing 12.5 µl 2x SYBR Green PCR Master Mix, 1 µl primers, 2 µl cDNA, and 8.5 µl of RNase Free Water. The primers for the target and internal reference (β-actin) genes obtained from Metabion (Germany) were 5'-CACCAGCTCTGAACAGATCATGA-3' and 5'-TCAGCCCATCTTCTTCCAGATGGT-3' used for Bax, and those for BCL-2 were 5'-CACCCCTGGCATCTTCTCCTT-3' and 5'-AGCGTCTTCAGAGACAGCCAG-3' (Kinouchi, 2003), whereas those for ß-actin were 5'-TCCTCCTGAGCGCAAGTACTCT-3 and 5'-GCTCAGTAACAGTCCGCCTAGAA-3 (Banni *et al.,* 2010). Each cycle consisted of denaturing for 5 min at 94 °C, annealing for 30 s at the appropriate annealing temperature, and polymerization for 30 s at 72 °C. The dissociation stage was added after amplification to verify the specificity of the PCR products, quantitative analysis was performed with Stratagene MX3005P software, and variations in gene expression on the mRNA of the different samples were estimated according to the "ΔΔCt" method (Yuan, et al., 2006).

Statistical analysis

Statistical analysis was performed using the IBM SPSS version 25 software package (SPSS, IBM, and Chicago, IL, USA). An independent t test was used to compare the quantitative results, and the correlation coefficient was also used to study the relationships between the quantitative biochemical variables.

RESULTS

Oxidative stress biomarkers

Compared to the control group G1, Abamectin- intoxication (ABA) after 10 days of exposure during lactation periods revealed a significant increase in brain protein carbonyl levels ($p<0.001$) and a decrease in GSH content ($p<0.01$), while the antioxidant enzymes GP_x ($p<0.01$) and GST ($p<0.05$) activities were increased markedly. However, brain SOD activity ($p<0.01$) was significantly decreased (Table 1). The same trend of oxidative stress biomarkers disturbance was noticed after the lactation period (21 days), although the exposure was stopped in the middle of the lactation period (Table 2).

Gene expression

The gene expression of apoptosis biomarkers (BCL-2 and BAX) is presented in Table 3. The data revealed that exposure to abamectin at a dose level of 0.211 mg/kg (1/100 of LD_{50}) during the lactation period from PND1 to PND10 induced a significant increase in apoptosis levels, as evidenced by the rise in BAX expression (a pro-apoptotic marker) fourfold-fold after ten days of exposure, while BCL-2 expression (an anti-apoptotic marker) decreased significantly to 50% of the control in pub's brain. The same trend was noticed in the expression of BAX (3-fold upregulation) and BCL-2

Table 1. Oxidative stress biomarkers in neonatal brains exposed to abamectin after 10 days of exposure during the lactation period

Treatments	**SOD** (U/ml)	**GST** (nM/min/mg pro.)	**GP_x** (µmol/ml)	**PC** (nmol/ml)	**GSH** (µmol/ml)
G1: Control (5 mlD.W./kg)	4.707±0.391	36.25±0.725	5.082±0.087	6.945±0.237	1.729±0.044
G2: ABA (0.211 mg/kg)	3.172±0.260**	38.656±0.728*	5.377±0.080**	10.575±0.145***	1.529±0.006**

*Significant at 0.05 ** Significant at 0.01 *** Significant at 0.001.

Table 2. Oxidative stress biomarkers in the neonatal brain after the lactation period (21 days) of exposure to abamectin (PND1 to PND 10)

Treatments	**SOD** (U/ml)	**GST** (nmol/min/mg pro.)	**GP_x** (µmol/ml)	**PC** (nmol/ml)	**GSH** (µmol/ml)
G1: Control (5 mlD.W./kg)	3.666±0.629	44.843±0.939	3.709±0.262	16.03±0.49	1.705±0.016
G2: ABA (0.211 mg/kg)	2.787±0.086***	52.21±1.70**	3.334±0.063	19.30±0.470*	1.580±0.029***

*Significant at 0.05 ** Significant at 0.01 *** Significant at 0.001.

Table 3. Gene expression of BAX and BCL2 in the neonatal brain after 10 and 21 days of lactation exposure to abamectin (PND1 to PND 10)

Treatments	BAX		BCL2	
	10-days	21-days	10-days	21-days
Group G1: Control (5 mlD.W./kg)	1.01±0.033	1.00±0.052	1.01±0.06	1.01±0.017
Group G2:ABA (0.211 mg/kg)	4.37±0.17***	2.80±0.25***	0.52±0.06***	0.66±0.04***

*Significant at 0.05 ** Significant at 0.01 *** Significant at 0.001.

(66% downregulation) at the end of the lactation period (21 days), although exposure to abamectin was observed during the first ten days of the lactation period.

DISCUSSION

Excessive pesticide use on plants increases concern about environmental damage, and ingestion of specific products has a substantial neurotoxic effect (Franco *et al.,* 2010). Xenobiotic exposure at a young age has been shown to cause more severe abnormalities. Such susceptibility could be caused by insufficient excretory and xenobiotic–metabolizing systems in infants. Due to higher brain absorption of xenobiotics, the rapidly developing nervous system is particularly vulnerable (Nahas *et al.,* 2019). Glutamate is the brain's ubiquitous stimulant. It plays a role in memory, synaptic plasticity, learning, and cognition (Daghestani *et al.,* 2009). Inhibition of the neurotransmitter (GABA) receptor in mammals opens the ionotropic GABA-A receptor-gated Cl channels that are only found in the CNS (McCavera and Wolstenholme, 2007). Furthermore, the breakdown of hazardous compounds by CYP-2E1 (detoxification membrane protein) yields more reactive and toxic by-products, as oxidative stress plays a crucial role in abamectin-induced toxicity. Such oxidative stress reactions were observed in the livers of abamectin-intoxicated rats. Furthermore, following abamectin exposure, brain redox indicators were altered (Radi *et al.,* 2020).

Alterations in the development process may occur during the postnatal stage, resulting in greater neuronal cell degeneration (Barkur and Bairy, 2016). Apoptosis occurs during various developmental stages, including neurulation, synaptogenesis, and elimination of adult neurons. Apoptosis is likely to occur in neurons that do not reach their appropriate new target throughout development due to a lack of enough neurotrophic factors necessary for CNS growth and development (Czabotaret *et al.,* 2014).

The present study revealed that exposure to abamectin at a dose level of 0.211 mg/kg (1/100 of LD_{50}) during the lactation period from PND1 to PND10 induced a significant increase in apoptosis levels, as evidenced by the rise in BAX gene expression exposure and a decline in BCL-2 gene expression in the pub's brain. The induction of apoptosis in the brains of pubs may occur by activation of the MAPK pathway (a signaling pathway is involved in practically every biological activity when generating ROS increases (Wada and Penninger, 2004 & McCubrey *et al.,* 2007). BAX controls the release of cytochrome c from mitochondria by constructing a mitochondrial pore. The release of cytochrome c from mitochondria during permeability transition and hypertrophy can promote the effects of oxygen free radicals by diminishing redox homeostasis (Votyakova and Reynolds, 2005). The same trend of apoptosis was noticed in TM3 cells after exposure to abamectin, which induces mitochondrial depolarization and apoptosis formation (Zhu *et al.,* 2020).

The interruption of the ratio between ROS generation and removal may result in cell damage, apoptosis, or even necrosis (Lushchak, 2011). The present study revealed that exposure to abamectin during lactation periods led to a significant increase in protein carbonyl levels in pup brains. Protein carbonylation arises either from amino acid oxidation by reactive oxygen species (ROS) or from the interaction of lipid peroxidation products with amino acids (Zheng and Bizzozero, 2010). Protein carbonyl has long been utilized as a metric of oxidative stress levels in various neurodegenerative pathologies, including multiple sclerosis (Bizzozero *et al.,* 2005) and Alzheimer's disease (Sultana and Butterfield, 2010).

A glutathione decline below a specific level was noticed in the present study after exposure to abamectin during lactatation periods, which is connected with permeability friction. This action results in increased superoxide formation, either due to an increase in ubiquinone redox cycling within complex III or as a result of an increase in ubiquinone redox cycling outside of complex III (Chen, *et al.* 2003). Glutathione depletion leads to H_2O_2 buildup and cell injury since it is the primary antioxidant responsible for eliminating ROS generation (Radi *et al.,* 2020).

Abamectin treatment significantly impacted the activities of SOD, GPx and GST in the brains of pups in the present study. As a result, the effects of abamectin on the ROS removal pathway appear to be the primary cause of abamectin-induced oxidative stress. Such ef-

fects were documented in MEFs by Liang *et al.* (2020). The obtained results were in the same direction as those of Zhang *et al. (*2016 & Zhang *et al.,* 2017), where abamectin treatment caused apoptosis and DNA damage in human HepG2 cells. Additionally, hepatocyte apoptosis of juvenile fish after abamectin exposure is probably triggered by ROS generation even at a much lower concentration than the safe concentration and the realistic environmental levels (Honga *et al.,*2020).

This study revealed the impact of abamectin on developmental neurotoxicity in the brains of neonates whose mothers were exposed to these pesticides during critical periods of development. The results exhibited a significant change in oxidative stress biomarkers and apoptosis gene expression. Pesticides and other lipophilic harmful pollutant particles have been found in the mother's adipose tissue, and these particles are transmitted to the infant *via* breast milk, generating congenital deficits and negative impacts as reported earlier Mortuza *et al.,* (2019). The neonatal neural system is five times more vulnerable than the adult nervous system, as evidenced by Aaseth *et al.* (2020) and Iqubal *et al.* (2020).

Conclusion

Finally, exposure to abamectin during critical periods of development, such as lactation periods, resulted in a significant change in oxidative stress biomarkers and apoptosis gene expression in the brains of rat neonates whose mothers were exposed to these pesticides. and these particles are transmitted to the infant *via* breast milk, generating congenital deficits and negative impacts.

Institutional review board statement: The Institutional Review Board (IRB), particularly the Institutional Animal Care and Use Committee, Zagazig University, Zagazig, Egypt (ZU-IACUC/2/F/78/2020), reviewed and approved the study design and experimental protocols.

Conflict of interest

The authors declare that he has no conflicts of interest.

REFERENCES

1. Aaseth J, Wallace DR, Vejrup K. & Alexander J (2020): Methylmercury and developmental neurotoxicity: A global concern. *Current Opinion in Toxicology,* 19: 80-87. https://doi.org/10.1016/j.cotox.2020.01.005
2. Bai, S. H. & Ogbourne, S. (2016). Ecotoxicological effects of the avermectin family with a focus on abamectin and ivermectin. *Chemosphere,* 154, 204-214.https://doi.org/10.1016/j.chemosphere.2016.03.113
3. Banni, M.; Messaoudi, I.; Said, L.; El Heni, J.; Kerkeni, A. & Said, K. (2010): Metallothionein gene expression in the livers of rats exposed to cadmium and supplemented with zinc and selenium. *Archives of environmental contamination and toxicology,* 59(3): 513-519. https://doi:10.1007/s00244-010-9494-5
4. Barkur, R.R. & Bairy, L.K. (2016). Histological study on hippocampus, amygdala and cerebellum following low lead exposure during prenatal and postnatal brain development in rats. *Toxicology and industrial health,* 32(6): 1052-1063. doi:10.1177/0748233714545624
5. Bizzozero, O. A.; DeJesus, G.; Callahan, K. & Paruszyn, A. (2005): Elevated protein carbonylation in the brain white matter and gray matter of patients with multiple sclerosis. *Journal of neuroscience research,* 81(5): 687-695. https://doi:10.1002/jnr.20587
6. Bradford, M. M. A (1976). Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry,* 72(1-2): 248-254. https://doi.org/10.1016/0003-2697(76)90527-3
7. Chen Q., Vazquez E.J., Moghaddas S., Hoppel C.L. & Lesnefsky E.J. (2003): Production of reactive oxygen species by mitochondria: central role of complex III. *Journal of Biological Chemistry,* 278(38): 36027-36031. https://doi:10.1074/jbc.M304854200
8. Council, N. R.,: Guide for the care and use of laboratory animals, National Academies Press. Washington, DC. [Google Scholar, (2011)
9. Czabotar P, Lessene G, Strasser A & Adams J (2014): Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy *Nature reviews Molecular cell biology,* 15(1): 49-63. https://doi:10.1038/nrm3722
10. Daghestania , M. H., Selimb , M. E., Abd-Elhakimc, Y. M., Saidd , E. N., Abd El-Hameede , N. E. Khalilc , S. R. and El-Tawilf . O.S. (2017): The role of apitoxin in alleviating propionic acid-induced neurobehavioral impairments in rat pups: the expression pattern of Reelin gene. *Biomedicine & Pharmacotherapy,* 93: 48-56. doi:10.1016/j.biopha.2017.06.034
11. Danaher, M. Radeck, W. Kolar, L. Keegan, J. Cerkvenik Flajs, V & Curr Pharm Biotechnol (2012): Recent developments in the analysis of avermectin and milbemycin residues in food safety and the environment. *Current Pharmaceutical Biotechnology,* 13(6), 936-951. https://doi: 10.2174/138920112800399068
12. Ellman, G.L. (1959), Tissue sulfhydryl groups. *Archives of biochemistry and biophysics,* 82(1): 70-77. https://doi:10.1016/0003-9861(59)90090-6
13. Franco R., Li S., Rodriguez-Rocha H., Burns M. & Panayiotidis M.I. (2010**):** Molecular mechanisms of pesticide-induced neurotoxicity: Relevance to Parkinson's disease. *Chemico-Biological Interactions,* 188(2), 289-300. doi: 10.1016/j.cbi.2010.06.003
14. Gonza´lez-Garcı´a M., Thompson C.B. & Ding L., (1994): Surface analysis of titanium electrodes. *Applied Surface Science,* 78(4), 457-466. https://doi:10.1016/0169-4332(94)90069-8
15. Habig, W.H., Pabst, M.J. & Jakoby, W.B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry,* 249(22): 7130-7139.
16. Heyer, D.B. & Meredith, R.M. (2017). Environmental toxi-

cology: Sensitive periods of development and neurodevelopmental disorders. *Neurotoxicology,* 58: 23-41. https:// doi: 10.1016/j.neuro.2016.10.017

17. Honga, Y. Huanga, Y. Yangb , X., Zhanga , J., Lia , L. Huanga , Q. & Huang Z. (2020): Abamectin at environmentally realistic concentrations causes oxidative stress and genotoxic damage in juvenile fish (Schizothorax prenanti). *Aquatic Toxicology,* 225: 105528. https:// doi: 10.1016/j.aquatox.2020.105528
18. Iqubal, A. Ahmed M., Ahmad S., Sahoo, C. R. Iqubal M. K. & Haque S. E. (2020): Environmental neurotoxic pollutants. *Environmental Science and Pollution Research,* 1-24. https:// doi:10.1007/s11356-020-10539-z
19. Jurewicz, J. & Hanke, W. (2008). Prenatal and childhood exposure to pesticides and neurobehavioral development: review of epidemiological studies. *Int J Occup Med Environ Health,* 21(2), 121-132. https:///doi: 10.2478/v10001-008-0014-z
20. Kinouchi, S. (2003). Changes in apoptosis-related genes (Bcl-2, Bax) in the urethras of old female rats following estrogen replacement. *Yonago Acta Medica,* 46(4): 109-115.
21. Liang, Y. Dong, B. Pang N. & Hu J. (2020): Abamectin induces cytotoxicity via the ROS, JNK, and ATM/ATR pathways. *Environmental Science and Pollution Research,* 27(12): 13726-13734. https://doi: 10.1007/s11356-019-06869-2
22. Liou C.M., Tsai S.C., Kuo C.H., Ting H. & Lee S.D. (2014): Cardiac Fas-dependent and mitochondria-dependent apoptosis after chronic cocaine abuse. *International journal of molecular sciences,* 15(4): 5988-6001. https://doi.org/10.3390/ijms15045988
23. Lushchak, V.I., (2011). Environmentally induced oxidative stress in aquatic animals. *Aquatic toxicology,* 101(1): 13-30. https:// doi:10.1016/j.aquatox.2010.10.006
24. Marklund, S. & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European journal of biochemistry,* 47(3): 469-474. https:// doi: 10.1111/j.1432-1033.1974.tb03714.x
25. Mattson, M.P., (2006). Neuronal life-and-death signaling, apoptosis, and neurodegenerative disorders *Antioxidants & redox signaling,* 8(11-12), 1997-2006. https://doi:10.1089/ars.2006.8.1997
26. McCavera, S., T. Walsh & Wolstenholme, A. (2007). Nematode ligand-gated chloride channels: an appraisal of their involvement in macrocyclic lactone resistance and prospects for developing molecular markers. *Parasitology,* 134(8): 1111-1121. https:// doi.org/10.1017/S0031182007000042
27. McCubrey., J.A. Steelman, L. S. Chappell, W. H. Abrams, S. L. Wong, E. W.T. Chang, Lehmann, F. Terrian, B. Milella, D. M., Tafuri, A., Stivala, F. Libra,M., Basecke, J., Evangelisti, C., Martelli, A. M. & Franklin R. A.(2007): Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research,* 1773(8): 1263-1284. https://doi: 10.1016/j.bbamcr.2006.10.001
28. Mortuza T.B., Edwards G.L., White C.A., Patel V., Cummings B.S. & Bruckner J.V. (2019).: Age dependency of blood–brain barrier penetration by cis-and trans-permethrin in the rat. *Drug Metabolism and Disposition,* 47 (3): 234-237. https://doi.org/10.1124/dmd.118.084822
29. Muñoz-Pinedo, C. (2012). Signalling pathways that regulate life and cell death: evolution of apoptosis in the context of self-defence. *Self and Nonself,* 124-143. https:// doi:10.1007/978-1-4614-1680-7_8
30. Nahas, A. A., Ziada, R., M. Gamila, Kotb, A. M. & Ahmed Farag A. Gh. (2019): Oxidative Stress and Apoptosis Biomarkers in Neonate Rats' Brain Exposed to Diquat during Lactation. *Egyptian Journal of Hospital Medicine*, 77 (6). https:// doi: 10.21608/EJHM.2019.67316
31. N'Go P.K., Azzaoui F.Z., Ahami A.O.T., Soro P.R., Najimi M. & Chigr F. (2013): Developmental effects of malathion exposure on locomotor activity and anxiety-like behavior in Wistar rats. *Health,* **5**(3), 603-611. https:// doi:10.4236/health.2013.53A080
32. Necheles, T. F.; N. Maldonado; A. Barquet C. & Allen D. M. (1969): Brief report: Hemozygous erythrocyte glutathione-peroxidase deficiency: *clinical and biochemical studies. Blood,* 1969. 33(2): 164-169. https://doi.org/10.1182/blood.V33.2.164.164
33. Olney, J.W Tenkova, T. Dikranian, K.Qin, Y.-Q.Labruyere, J. & Ikonomidou C. (2002)**:** Ethanol-induced apoptotic neurodegeneration in the developing C57BL/6 mouse brain. *Developmental Brain Research,* **133**(2), 115-126. https://doi: 10.1016/s0165-3806(02)00279-1
34. Omura, S. (2008). Ivermectin: 25 years and still going strong. *International journal of antimicrobial agents*, 31(2), 91-98. https:// doi: 10.1016/j.ijantimicag.2007.08.023
35. O'Rahilly, R. & Müller, F., (2008). Significant features in the early prenatal development of the human brain. *Annals of Anatomy-Anatomischer Anzeiger,* 190(2): 105-118. https://doi:10.1016/j.aanat.2008.01.001
36. Radi, A. M. Mohammed, E T. Abushouk, A.I. Aleya L & Abdel-Daim M. M. (2019): The effects of abamectin on oxidative stress and gene expression in rat liver and brain tissues: modulation by sesame oil and ascorbic acid. *Science of The Total Environment,* 701: 134882. https:// doi: 10.1016/j.scitotenv.2019.134882
37. Reznick, A.Z. & Packer, L. (1994) [38] Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Methods in Enzymology,* 233: 357-363. https:// doi:10.1016/s0076-6879(94)33041-7
38. Seneff, S., N. Swanson, & Li C. (2015). Aluminum and Glyphosate Can Synergisti-cally Induce Pineal Gland Pathology: Con-nection to Gut Dysbiosis and Neurological Disease. *Agricultural Sciences,* 6(01), 42. https:// doi: 10.4236/as.2015.61005
39. Sokolova I.M., Frederich M., Bagwe R., Lannig G. & Sukhotin A.A. (2012): Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Marine environmental research,* 79: 1-15. https://doi: 10.1016/j.marenvres.2012.04.003
40. Sultana, R. & Butterfield, D.A. (2010). Role of oxidative stress in the progression of Alzheimer's disease. *Journal of Alzheimer's Disease,* 19(1): 341-353. https:// doi: 10.3233/JAD-2010-1222
41. Votyakova, T.V. & Reynolds, I.J. (2005). Ca^{2+} induced permeabilization promotes free radical release from rat brain mitochondria with partially inhibited complex I. *Journal of Neurochemistry,* 93(3): 526-537. https:// doi:10.1111/j.1471-4159.2005.03042.x
42. Wada, T. and Penninger, J.M. (2004). Mitogen-activated

protein kinases in apoptosis regulation. *Oncogene,* 23 (16): 2838-2849. https://doi:10.1038/sj.onc.1207556

43. Yang, C.-C. (2012). Acute human toxicity of macrocyclic lactones. *Current Pharmaceutical Biotechnology*, **13**(6), 999-1003. https://doi:10.2174/138920112800399059
44. Youle, R.J. & Strasser, A.(2008). The BCL-2 protein family: opposing activities that mediate cell death. *Nature reviews Molecular Cell Biology,* **9(**1): 47-59. https://doi: 10.1038/nrm2308
45. Yuan, J.S.; Reed, A.; Chen, F. & Stewart, C.N. (2006): Statistical analysis of real-time PCR data. *BMC Bioinformatics,* 7(1): 1-12.
46. Zhang, Y., Luo, M., Xu, W., Yang, M., Wang, B., Gao, J., Li, Y. & Tao, L., (2016): Avermectin confers its cytotoxic effects by inducing DNA damage and mitochondria-associated apoptosis. *Journal of Agricultural and Food Chemistry,* 64(36): 6895-6902. https://doi:10.1021/ acs.jafc.6b02812
47. Zhang, Y., Wu, J., Xu, W., Gao, J., Cao, H., Yang, M., Wang, B., Hao, Y. and Tao, L. (2017): Cytotoxic effects of avermectin on human HepG2 cells in vitro bioassays. *Environmental Pollution,* 220: 1127-1137. https:// doi:10.1016/j.envpol.2016.11.022
48. Zheng, J. & Bizzozero, O.A. (2010). Accumulation of protein carbonyls within cerebellar astrocytes in murine experimental autoimmune encephalomyelitis. *Journal of Neuroscience Research,* 88(15): 3376-3385. https:// doi:10.1002/jnr.22488
49. Zhu, S., Zhou J., Zhou Z. & Zhu Q. (2019): Abamectin induces apoptosis and autophagy by inhibiting reactive oxygen species-mediated PI3K/AKT signaling in MGC803 cells. *Journal of Biochemical and Molecular Toxicology*, 33(7), e22336. https:// doi:10.1002/jbt.22336
50. Zhu, S., Zhou J., Sun X., Zhou Z. & Zhu Q. (2020): ROS accumulation contributes to abamectin□induced apoptosis and autophagy via the inactivation of PI3K/AKT/mTOR pathway in TM3 Leydig cells. *Journal of Biochemical and Molecular Toxicology,* 34(8): e22505. https://doi: 10.1002/ jbt.22505
51. Zhu, W.J., Li, M., Liu, C., Qu, J.P., Min, Y.H. & Xu, S.W. (2013): Avermectin induced liver injury in pigeon: mechanisms of apoptosis and oxidative stress. *Ecotoxicology and Environmental Safety,* 98, 74-81. https://doi:10.1016/ j.ecoenv.2013.09.021