

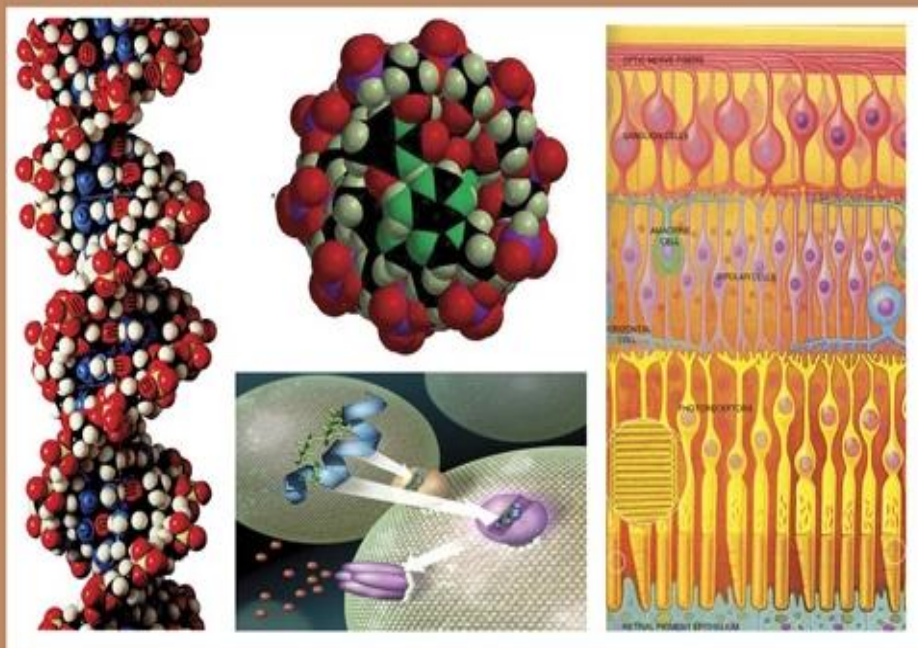


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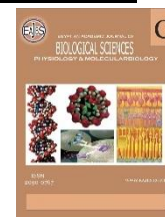
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## Neurotoxic and Oxidative Stress Impacts in Male Albino Rats Exposed to Single Dose of Abamectin

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### ABSTRACT

**Background:** The widespread use of pesticides in public health and agriculture operations has led to significant environmental degradation and potential health risks. It's worth noting that the main reason for toxicity with most pesticides, especially insecticides like is to attack nervous system components. **Aim:** this study achieved to study the effect of single oral and dermal sub-lethal exposure of abamectin as a neurotoxic compound on acetylcholinesterase (AChE) and oxidative stress biomarkers in male albino rat's brains. **Methods:** experimental rats were divided into three groups as control, oral and dermal (15 rat each). The tested dose was  $\frac{1}{2}$  of LD<sub>50</sub> for oral and dermal experiments. **Results:** single abamectin exposure by oral or dermal is connected with neurotoxicity in exposed rats as evidenced by a marked reduction in. Acetylcholinesterase (AChE) activity and induction of oxidative stress. The oxidative stress biomarkers lipid and protein oxidation (MDA and PC) were significantly increased. While antioxidant biomarkers, glutathione (GSH) glutathione peroxidase (GPx) and superoxide dismutase (SOD) were markedly declined in both oral and dermal exposure. GST was increased markedly in brain tissues. **Conclusion:** this study through light on neuro and oxidative impact of abamectin after a single exposure (acute effect). SO, awareness of the importance of responsible use of pesticides must be implemented for reducing the hazard impact of agrochemicals.

### INTRODUCTION

Bio-pesticides among other agrochemicals are widely employed in agriculture to eradicate pests that destroy crops and promote sustainable development (Adu *et al.*, 2019). These compounds have the potential to be harmful to some creatures, including persons, and therefore must be handled and safely disposed of (WHO, 2016). Pesticide exposure has been linked to cancer, neurologic and developmental problems, lung abnormalities (Mostafalou and Abdollahi, 2017), endocrine disruption (Maqbool *et al.*, 2016; and Nascimento *et al.*, 2018) systemic inflammation and immunological dysfunction in humans (Karami-Mohajeri and Abdollahi 2013; and Mokarizadeh *et al.*, 2015).

Studying the pathways of pesticide and metal impacts on the hematological, biochemical, and immunological systems can aid in the prevention of long-term diseases in farmers and improve the quality of life for rural workers. Neurotoxic effects have been considered as the second most harmful pesticide toxicity. It's worth noting that the main reason for toxicity with most pesticides, especially insecticides like is to attack nervous system components (Abdollahi and Karami-Mohajeri 2012; and Karami-Mohajeri *et al.*, 2013).

The widespread use of pesticides in public health and agriculture operations has led to significant environmental degradation and potential health risks. Pesticides' toxicity varies depending on the types of exposure, such as dermal, oral, or inhalation exposure. Aware of the harmful nature of the chemical, the risk of pesticide exposure tends to grow with dose (concentration) and basic durations. (Meenakshi *et al.*, 2012). Exposure to pesticides can occur in a variety of ways, including technical jobs the manufacturing, distribution, application, and use of agricultural chemicals. Sudden incidents are another common source of contamination for applicators, and the rates of exposure associated with these events can have both short- and long-term health consequences (MacFarlane *et al.*, 2013)

The most frequently used substance, abamectin which is used for both agricultural and medicinal objectives and has attracted interest due to its high toxicity to non-target species. (Bai and Ogbourne, 2016). Abamectin is a very potent and a key component of many public health services aimed at preventing the spread of lymphatic filarial illness and ultimately eliminating it worldwide. (Nasr *et al.*, 2016). Due to its strong lipophilic nature, abamectin has been found to aggregate at elevated amounts in fatty tissue, such as the liver and fat (Gonzalez

*et al.*, 2009). Insects are harmed by abamectin, and mammals may be affected by it. abamectin poisoning destroyed 57 calves over the course of four years. Because the severity of intoxication symptoms varies, many animals recover quickly. This is thought to be due to an incorrect dosage given to the animals (Castanha Zanoli *et al.*, 2012). Using a commercial formulation, researchers discovered indications of abamectin liver damage, including increased AST activity in rats, fed doses corresponding to 1/10 or 1/100 of the LD<sub>50</sub> (18 mg/kg) in the diet for 30-days (Eissa and Zidan, 2010).

Acetylcholinesterase (AChE) has been employed as a biomarker for pesticide toxicity. This enzyme is required for the proper functioning of the human central nervous system. It's in the responsibility of precise regulation, modulation of neurotransmitter release, and the acetylcholine hydrolysis process at cholinergic synapses, as well as identifying environmental pollutants. Anticholinesterase chemicals like organophosphorus pesticides and carbamate, as well as several toxic substances, suppress AChE, making it an acceptable enzymatic biomarker of neurotoxicity. (Zhao *et al.*, 2015).

On a molecular scale, toxicants trigger a range of cytotoxicity pathways, including oxidative stress appearing to be the common element, leading to damage to cellular membrane lipids, DNA, and proteins. As well as antioxidant enzyme modification are likely to induce injury to any kind of molecule inside the cell, such as polyunsaturated fatty acids, glutathione, certain amino acids, and so on, due to their high reactivity (Poljšak and Fink 2014). Oxidative stress is a key factor in avermectins-induced cytotoxicity (Zhu *et al.*, 2013). Oxidative stress, immunological damage, cytotoxicity and genotoxicity induced by abamectin in mammals, birds and fish (Huang *et al.*, 2019 and Srivastava, *et al.*, 2020).

So, the aim of this study is to identify the impact of bio-pesticides (abamectin) through dermal or oral exposure on acetylcholinesterase and oxidative stress biomarkers in brain tissues of male albino rats during the acute toxicity period.

## MATERIALS AND METHODS

### Insecticide:

A commercial formulation of abamectin (1.8 % EC) was supplied by Central Agricultural of Pesticides Laboratory (CAPL), Agricultural Research Centre (ARC), Dokki, Giza, Egypt.

### Experimental Design:

Forty-five rats aged 3–4 months and weighing 160-180 g were purchased from the breeding unit of the Mammalian and Aquatic Toxicology Department, CAPL, ARC, Dokki, Giza, Egypt. The rats were kept in polypropylene cages with five rats per cage and allowed to acclimatize for one week under normal conditions of 20-25°C, percent relative humidity (50-55%), and a 12/12 h photoperiod (light/dark cycles). The rats were given unlimited access to a normal pellet diet and water. The animals were divided into three groups, randomly (15 rats each). The first group of 10 rats acted as a control group, getting no treatments. oral exposure was performed on the second group at the dose of 10 mg/ kg (1/2 LD<sub>50</sub>). While the third group was treated with cutaneous exposure at the dose of 150 mg/ kg (1/2 LD<sub>50</sub>). The trunks (10 percent of the skin surface) of the experimental rats are clipped using electric clippers (Oster Corp., Model A2, Milwaukee, WI, USA) before 24 hours of the dermal exposure of insecticide. The trunk of the animal treated with abamectin is wrapped with gauze and rubber damming. The gauze is removed twenty-four hours after application of

## RESULTS

### Abamectin and Neurotoxic Biomarker Acetylcholinesterase (AChE):

The results (Table, 1) showed an increase significantly ( $p \leq 0.001$ ) in AChE activity in the rat's brain treated with

insecticide, and the treated sites are washed with sterile distilled water. Daily observations and responses are documented for 14 days. the rats are euthanized by decapitation after 1, 7 and 14 days of treatment to obtain the brain which is washed with cold saline buffer and immediately stored at -80 °C. This study was conducted in accordance with ethical procedures and policies approved by the Institutional Animal Care and Use Committee of Zagazig University (No. ZUIACUC/2/F/38/2019).

### Tissue Preparation:

The brain tissues were homogenised in an ice-cold 50 mM sodium phosphate buffer (pH 7) with 0.1 mM ethylenediaminetetraacetic acid (EDTA), giving a 10% (W/V) homogenate. The homogenates were spun for 30 minutes at 4°C at 12,000 rpm. the supernatant was collected and utilized to investigate enzyme activity.

### Neurological and Oxidative Stress Biomarkers Assay:

Acetylcholinesterase (AChE) activity was assayed in liver tissues by the method of Ellman, *et al.*, (1961). Total protein (TP) level was quantified by the procedure of Bradford (1976). Lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive products (malondialdehyde, MDA) (Ohkawa, *et al.*, 1979). Protein carbonyl (PC) content was assayed using the mentioned method Yan *et al.*, (1995). Superoxide dismutase (SOD) and glutathione -S- transferases (GST) activity was measured by the methods of Marklund and Marklund, (1994) and Habig, *et al.*, (1973) respectively. Total reduced glutathione (GSH) content and glutathione peroxidase activity were measured by Beutler, *et al.*, (1963) method.

abamectin by oral and dermal compared to the untreated group after 1 day of treatment. However, AChE inhibited significantly ( $p \leq 0.001$ ) after 7 and 14 days of exposure to abamectin dermally.

**Table (1):** Abamectin and acetylcholinesterase (AChE) as a neurotoxic biomarker.

Biomarkers	E.R.	Control	1-day	7-Days	14-Days
AChE (nmol/min/mg pro)	D.E.	617.17±23.4	787.40±26.08***	417.17±13.78***	413.87±14.83***
	O.E.		704.08±16.59**	527.88±19.20**	619.01±21.68

E.R.: Exposure Route, D.E.: Dermal Exposure, and O.E.: Oral Exposure. Data expressed as mean ±S.E.

\*: Significant at 0.05, \*\*: Significant at 0.01: and \*\*\*: Significant at 0.005.

### Abamectin and Oxidative Stress Biomarkers:

A significant increase in brain MDA levels was noticed in all groups ( $p \leq 0.001$ ) except, a significantly decreased after 1 and 14 days from treatment with a single dose by dermal and oral respectively, was observed. In addition, there was a significant increase in brain PC levels in groups treated with a sub-lethal dose of abamectin by gavage and

topical after all periods of exposure compared to the untreated group ( $p \leq 0.001$ ). Moreover, a significant increase in brain glutathione (GSH) content was seen in orally treated with abamectin after 7 days of exposure compared to the control group while a significant decrease was observed after dermal exposure after 7 and 14 days of treatment and after 14 days orally treated with abamectin (Table 2).

**Table (2):** Abamectin and oxidative stress biomarkers (MDA, PC and GSH)

Biomarkers	E.R.	Control	1-day	7-Days	14-Days
MDA ( $\mu\text{mol}/\text{mg pro}$ )	D.E.	17.87±0.77	18.57±0.66	47.48±1.44***	24.27±0.64***
	O.E.		26.49±0.79***	19.47±0.49	20.75±3.0.3***
PC (nmol/mg pro)	D.E.	29.38±1.13	56.11±1.3***	62.27±1.34***	38.56±0.92***
	O.E.		67.95±2.38***	56.03±0.65***	40.21±1.12***
GSH (nmol/mg pro)	D.E.	4.41±0.04	4.27±0.08	4.31±0.16*	3.66±0.14***
	O.E.		5.43±0.13***	4.39±0.14	3.45±0.14***

E.R.: Exposure Route, D.E.: Dermal Exposure, and O.E.: Oral Exposure. Data expressed as mean ±S.E.

\*: Significant at 0.05, \*\*: Significant at 0.01: and \*\*\*: Significant at 0.005.

### Abamectin and Antioxidant Enzymes Biomarkers:

A significant decrease in the activities of brain GPx was observed among rats treated with abamectin ( $p \leq 0.001$ ) by the two routes of exposure after all periods compared to the control group. The activity of SOD was inhibited significantly after oral exposure with

abamectin during the study periods. However, SOD significantly increased after 1 day of dermal treatment. GST activity was statistically increased in rat's brain treated dermally with abamectin after all periods and after 1 day in the group treated orally with abamectin (Table 3).

**Table (3):** Abamectin and antioxidant enzymes biomarkers (GP<sub>x</sub>, SOD and GST)

Biomarkers	E.R.	Control	1-day	7-Days	14-Days
GP <sub>x</sub> (nmol/min/mg pro)	D.E.	14.13±0.28	12.73±0.0.36***	12.19±0.37***	13.95±0.0.29***
	O.E.		10.16±0.28***	10.2±0.31***	14.13±0.16
SOD (U/mg pt)	D.E.	4.41±0.1	4.80±0.0.07**	4.21±0.0.12	4.63±0.04
	O.E.		3.74±0.12	3.67±0.04***	3.95±0.0.06***
GST (nmol/min/mg pro)	D.E.	8.51±0.0.37	12.27±0.50***	10.80±0.18**	17.20±0.70***
	O.E.		9.48±0.10*	8.67±0.28	9.23±0.21

E.R.: Exposure Route, D.E.: Dermal Exposure, and O.E.: Oral Exposure. Data expressed as mean ±S.E.

\*: Significant at 0.05, \*\*: Significant at 0.01: and \*\*\*: Significant at 0.005.

## DISCUSSION

Human exposure to pesticides can happen in many different ways, which include workers who perform pesticide manufacturing, transportation, delivery, and application. Residing in areas with high contaminants, pesticide can buildup in the food chain (Mostafalou and Abdollahi 2017)

Farmers who used agrochemicals were typically less aware of the negative health consequences of these chemicals, which might be explained by their lower educational level compared to the general population (Damalas and Eleftherohorinos, 2011). Pesticides from different chemical families, such as pyrethroids, triazines, and paraquat, might have an impact on AChE activity. (Lionetto *et al.*, 2013). As a result, AChE might be employed as an indication of exposure in the long-term monitoring of individuals exposed to pesticides other than organophosphate and carbamate (Hernandez *et al.*, 2005). Cell proliferation, maturation, and responsiveness to numerous insults, including stress affected by AchE activity. Also, it carries out an important role in non-enzymatic, trophic such as stimulation of neurogenesis and remodelling and neuromodulatory such promotion of long-term functional alterations in the central nervous system (Vučević *et al.*, 2009). The increase in AchE activity following both types of abamectin exposure after 1 day may be ascribed to an increase in Ach release by impacting sodium channels in cholinergic neurons, where abamectin, such as lindane, impacts GABA neurotransmitter synthesis (Vučević *et al.*, 2009). Malathion also results in an increase in hippocampal and cortical AchE activity after only a shorter time of exposure (Trevisan *et al.*, 2008).

Increased amounts of polyunsaturated fatty acids (PUFAs) in neuronal membranes also correlate to a higher oxygen consumption which results in the oxidative degradation of

polyunsaturated fatty acids found in cellular membranes. Their decomposition results in the production of cytotoxic and reactive malondialdehyde (MDA) that may disperse from its source to attack distant targets and form covalent connections with a variety of molecules. MDA also induced mitochondrial dysfunction in neurons by directly the production of reactive oxygen species (ROS) and altering mitochondrial proteins (Long *et al.*, 2009). Malondialdehyde (MDA) has been recognized as a redox status biomarker and is now regarded as a late oxidative stress biomarker end product (López *et al.*, 2007). MDA has a greater reactivity for nucleophiles such amino acids or protein) where, secondary detrimental reactions are induced by biomolecule interactions, resulting in biochemical changes (Ayala, *et al.*, 2014). The aggregation of modified or oxidized proteins in the brain causes the release of reactive oxygen species (ROS) and mitochondrial dysfunction, both of which are associated with neurodegeneration (Liu, *et al.*, 2017). Protein oxidation concludes specific amino acid modification, peptide cleavage and protein cross-linkage. Protein modification affects signal transduction, enzymes activity and proteolysis. (Kaur and Thakur, 2018).

Because pesticides may penetrate into the circulatory more rapidly through to the stomach than through the skin, the oral LD<sub>50</sub> is relatively low than the dermal LD<sub>50</sub> (Nesheim *et al.*, 2008). Furthermore, adjuvant substances used in pesticides to enhance biological functions as well as simplify implementation may reach target species contributing to the overall effect of pesticide exposure (Surgan *et al.*, 2010). These LD<sub>50</sub> values, on the other hand, must be adjusted to account for the concentration of the pesticide formulation in use. This is because the formulation has a major impact on the actual toxicity of a commercial pesticide product. For example, when a very toxic pesticide is

produced as an emulsifiable concentrate rather than a microcapsule solution, it becomes much more toxic (Damalas and Eleftherohorinos, 2011).

These findings are consistent with those of Nasr *et al.* (2016) who demonstrated that the brain antioxidant machinery was unable to counteract ABM-induced oxidative stress (SOD and CAT were dramatically decreased in rats treated with CPF, ABM, or both) attributable to the brain's high oxidative metabolism (Gandhi and Abramov, 2012). Furthermore, Li *et al.*, (2013) found that when the concentration of avermectin increased, the activities of SOD and CAT in the cerebellum decreased. Suppression of the free radical-scavenging SOD and CAT enzymes resulted in a buildup of superoxide, which enhanced lipid peroxidation and DNA modulation, disrupted gene expression, and cellular damage (Calviello *et al.*, 2006). Glutathione peroxidase (GSH-Px) is a wide class of selenium-containing enzymes that detoxify different peroxides by using the reduced form of glutathione as an electron donor. The availability of reduced glutathione and selenium levels is required for GSH-Px function. GSH-Px has the biological function of reducing the transformation of lipid hydroperoxides to their corresponding alcohols, as well as reducing free H<sub>2</sub>O<sub>2</sub>, protecting the tissue from oxidative damage (Baba *et al.*, 2016). The oxidative stress induced by abamectin intoxication may be related to the ability of abamectin to induce mitochondrial dysfunction and disturbance of calcium homeostasis (Maioli *et al.*, 2013). These results are similar to those found by Zhu *et al.*, (2013) and Li *et al.*, (2013). Whereas, avermectin caused liver damage, inhibition of SOD, and increased MDA levels. abamectin appears to have a significant impact on the expression of GABA receptors in rat brain tissue. macrocyclic lactones have a high affinity for glutamate-gated Cl Channels in neuronal cells localized to the CNS (McCavera *et al.*,

2007). Excessive chloride ions overburden the postsynaptic neuron, causing membrane hyperpolarization and inhibiting nerve signal transmission (Novelli *et al.*, 2012).

Finally, Integrated Crop Management (ICM), worker and product safety, usage of low acute and chronic toxicity pesticides, optimum formulation, safe packaging, simple application technique, and extended storage stability are all factors to consider in pesticides application for controlling the health hazards induced by pesticides (Chandler *et al.*, 2008)

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