

Degenerative effect of dimethyl disulfide on central neurons of cockroach *Periplaneta americana*Amina, E. Essawy^{1,2}, Jehan, M. Sorour^{1,2} and Abir, S. Al-Nasser¹¹Department of Biology, Faculty of Applied Sciences for Girls, Um Al Qura University, Makah, Saudi Arabia²Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt.amina_essawy@yahoo.com

Abstract: The present study was undertaken to evaluate the degenerative effect of dimethyl disulfide, extracted from *Allium porrum*, on the mesothoracic neurons of *Periplaneta americana*. Examination of the mesothoracic ganglia of male cockroach treated with 1/4 LC₅₀ of DMDS revealed severe ultrastructural alterations in their neurons. These alterations included: pyknotic nuclei, vacuolized cytoplasm with degenerated and indistinct organelles, Peripheral accumulation of multivesicular bodies and dilated and disorganized extracellular space. Degeneration of synaptic vesicles and swelling of the mitochondria were observed in axons of the neuropile. On the other hand, the nuclear envelope of glial cells nuclei showed deep invagination, while the cytoplasm appeared with abundant and aggregated mitochondria. These results indicate that DMDS has an insecticidal neurodegenerative effect and could be used in pest control.

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Key words: *Periplaneta americana*, DMDS, neurons, ultrastructural alterations.

1. Introduction

Since antiquity, plants and plant products have been shown to display not only their pharmacological benefits but other biological properties including pesticidal activities. Among these interesting plants *Allium spp.* show pesticidal effects (Ho *et al.*, 1996; Auger *et al.*, 2002, 2004; Dugravot *et al.*, 2002), most often linked to volatile substances derived from sulfur amino acids.

When cell membranes are ruptured, sulfur volatile compounds such as thiosulfates are released in the atmosphere. Depending on break down conditions, the resulting thiosulfates can lead to the formation of disulfides example Dimethyl disulfide (DMDS) tested in this study (Auger *et al.*, 1989; Dugravot, 2003).

Available data have shown that sulfur compounds in *Allium* can be classified not only as insecticides, acaricides, nematocides, herbicides, fungicides and bactericides, but also repellent against arthropods (Auger *et al.*, 2004; Ferry *et al.*, 2009).

Dugravot *et al.* (2003) stated that, sulfur compounds such as DMDS could represent a new alternative way to fight insect predators while avoiding the use of environmentally aggressive chemicals. In their physiological study on the neurotoxic effect of DMDS, Dugravot *et al.* (2002) showed that DMDS exerts insecticidal neurotoxicity through mitochondrial dysfunction and activation of insect KATP channels. However, to date, the degenerative effect of DMDS on the structure of insect neurons was rarely tackled.

Consequently, the present work was carried out essentially to evaluate the toxic effect of DMDS on the fine structure of neurons in the mesothoracic ganglia of cockroach.

2. Materials and Methods:**Experimental animals:**

Adult male Cockroaches *Periplaneta americana* were obtained from our laboratory stock colony and maintained at 25°C with a photoperiod of 12-hour light /12-hour dark.

The used chemicals:

Dimethyl disulfide (DMDS) with chemical formula C₂H₆S₂ was purchased from Sigma-Aldrich (ST Louis, MO, USA) and it was of the highest purity grade available (99%).

Experimental design:

Cockroaches were assigned into two groups with five pairs per group. Animals of the first group (control group) were placed for 24 hours in hermetically sealed glass jar and received no treatment. Animals of the second group (treated group) were kept in hermetically sealed glass jar containing ¼ LC₅₀ of DMDS (0.25 µl /L air) (Dugravot, 2002) and left for 24 hours.

Ultrastructural study:

After the end of the experiment, animals of both control and treated groups were dissected and mesothoracic ganglia were removed and immediately immersed in 4F1G mixture for two hours, then rinsed

in 0.1 M Phosphate buffer (PH 7.4). Fixed samples were post fixed in 1% OsO₄ for two hours at 4°C and washed with phosphate buffer for several times for ten minutes, dehydrated in graded ethanol, treated with propylene oxide and embedded in Epon. Ultrathin sections were cut on LKB Ultratome with uranyl acetate and lead citrate and examined by a Jeol 100CX electron microscope.

3. Results

Electron microscopic examination of mesothoracic ganglia of control cockroaches, revealed cortical neurons with large nuclei and finely dispersed chromatin. The cytoplasm of these neurons contains short pieces of rough endoplasmic reticulum rER, numerous rod-shaped mitochondria, cisternae of Golgi complex, free distributed ribosomes and electron dense secretory granules (Fig.1).

Numerous glial cells were observed surrounding the neurons and their axonal processes (Fig.2). They are characterized by large dense elongated nuclei with chromatin clumped at the periphery and thin cytoplasm containing mitochondria, rough endoplasmic reticulum rER and many ribosomes.

The neuropile of control mesothoracic ganglion (Fig.3) appeared with large number of axon terminals

containing mitochondria, microtubules, neurofilaments and different types of synaptic vesicles.

After exposure to ¼ LC₅₀ of DMDS severe ultrastructural alterations were recognized in nerve cells as well as in glial cells and axon profiles of treated insects.

Most nerve cells appeared necrotic with an irregular outline. Moreover, some nuclei appeared shrinkage with segregation of nucleolar components (Figs. 4, 5).The cytoplasm of these altered cells appeared vacuolized with degenerated and indistinct organelles (Fig.5). Peripheral accumulation of electron-dense granules as well as multivesicular bodies could be identified in the cytoplasm of some neurons (Fig.5). Also, some nerve cells were seen separated by a dilated and disorganized extracellular space (Figs.4, 6).

The glial cells nuclei showed deep invaginations of their nuclear envelope, while the cytoplasm appeared with abundant and aggregated mitochondria (Fig.7).

Many altered axons were observed with loss of its cytoplasmic texture and degeneration of synaptic vesicles. Swelling of the mitochondria and dilatation of their cristae were also recorded (Fig.8).

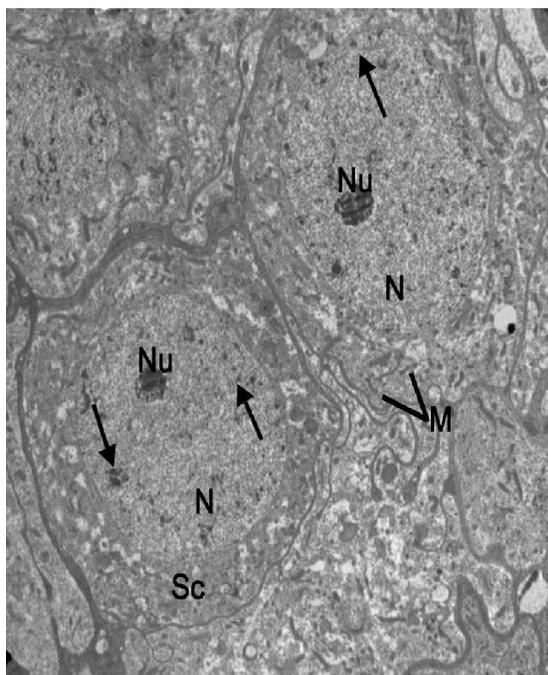


Fig.1: Electron micrograph of cortical neuron from control cockroach showing large spherical nucleus (N), nucleolus (Nu), dispersed chromatin (arrows), mitochondria (M) and secretory granules (Sc). X1500.

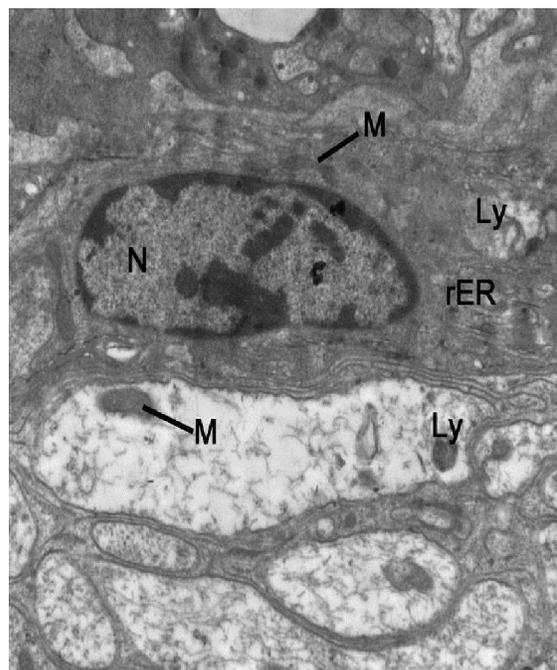


Fig.2: Electron micrograph showing normal glial cells. Ly: lysosomes, M: mitochondria, N: Nucleus, rER: rough endoplasmic reticulum. X5000.

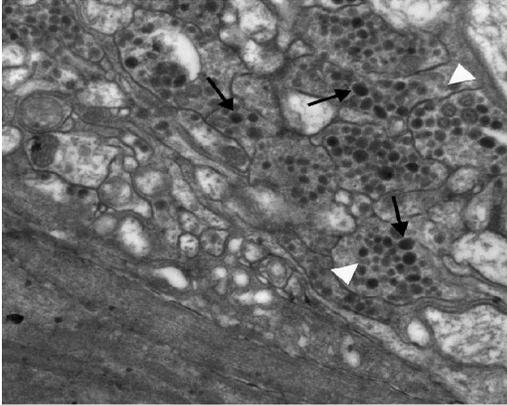


Fig.3: Electron micrograph from neuropile region of mesothoracic ganglia in control insect showing network of axons. Arrows pointed at electron-dense vesicles and head arrows indicate clear vesicles. X7500.

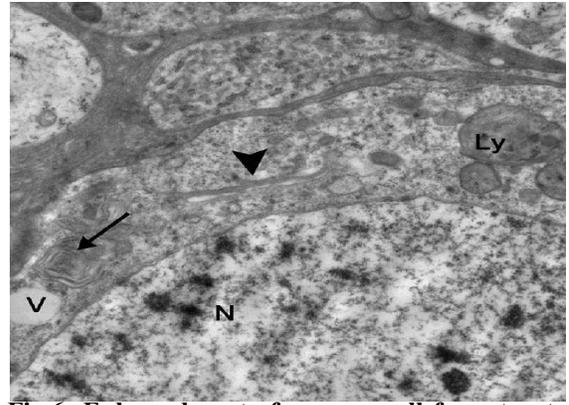


Fig.6: Enlarged part of a nerve cell from treated insect showing disorganized cytoplasm, myelin-like structures (arrow), Golgi bodies (head arrow), vacuoles (V), lysosomes (Ly) and nucleus (N).X5000.

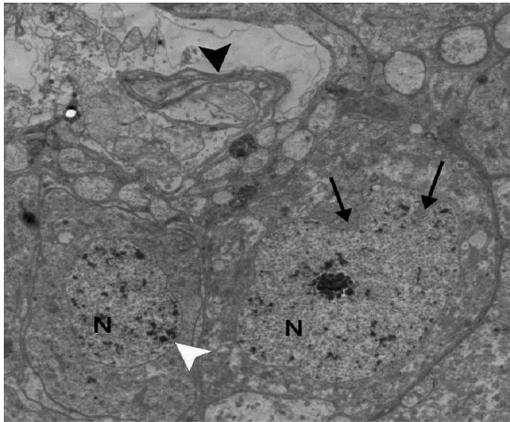


Fig.4: Electron micrograph of neurons in insect treated with DMSO. Note, irregular nuclear envelope (arrows), pyknotic nucleus (white head arrow), myelin-like structure (black head arrow).X1500.

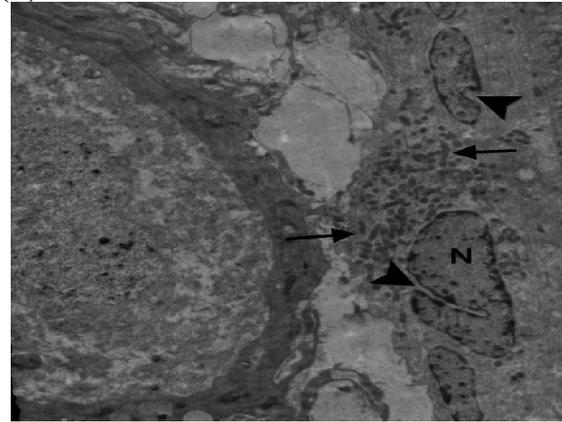


Fig.7: Glial cells of treated insect showing deep invagination of the nuclear envelope (head arrows), mitochondrial aggregation (arrows). X1500.

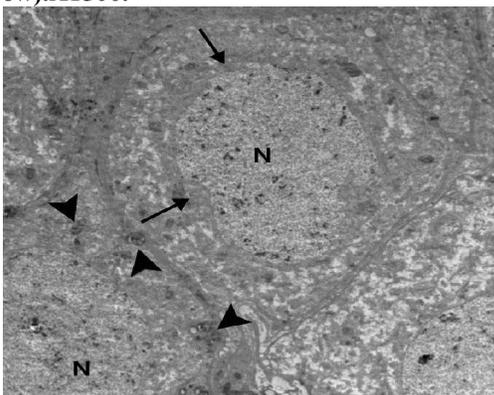


Fig.5: Electron micrograph of nerve cells of DMSO treated insect showing vacuolated cytoplasm, irregular nuclear envelope (arrows) and electron-dense granules (head-arrows). X1000.

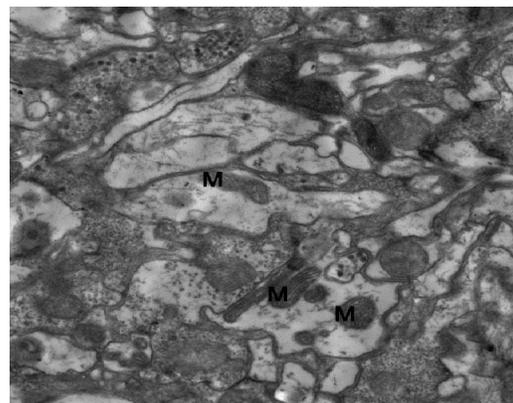


Fig.8: Electron micrograph of neuropile region in treated insect showing disorganization of some axons and axon profiles with swelling mitochondria (M) and degenerated vesicles. X5000.

4. Discussion

In the current study DMDS produced neuronal damage in the thoracic ganglia of *Periplaneta americana*. The lesions were observed in the nerve cells as well as in the glial cells in all treated insects.

The nerve cell nuclei appeared irregular, shrinkaged with segregated nucleolar components. The irregularly shaped nuclei resemble those observed in many insect cells due to toxicity (Braeckman and Raes, 1999) in Diptera and in Hemiptera (Sorour, 2001). Moreover, similar changes in the nuclei were reported in the neurons of buccal ganglia of land snails treated with carbamate pesticide (Essawy *et al.*, 2009,) or organotin compounds (Essawy *et al.*, 2011)

The changes of the nucleus and nucleolus could be attributed to the effect of the neurotoxin on the cytoskeleton of the affected neurons (Mc Ilwain and Hoke, 2005). While, the nucleolar segregation could be due to the inhibition of RNA synthesis resulting from a decrease in the activity of RNA polymerase which catalyzes the synthesis of RNA (Cmarko *et al.*, 2000).

In the present study the electron micrographs of treated insects showed vacuolization of nerve cell cytoplasm with indistinct organelles reflecting cell destruction. An apparent vacuolization in nerve cells and neurosecretory cells of suboesophageal ganglion was observed in *Spodoptera littoralis* larvae treated with the green chemical compound, spinetoram (Hamouda and Dahi, 2008) and in the cerebral ganglia of *B. glabrata* treated with Atrazine (Eissa and Omtan, 2007).

In addition, multivesicular bodies and electron-dense granules were observed in the cytoplasm of the nerve cells after treated the insects with DMDS.

The multivesicular bodies are a variety of heterolysosomes that behave as autolysosomes to digest endogenous material such as cell lysis products and secretory granules (Klionsky and Emr, 2000, Hamouda and Dahi, 2008).

Concerning the presence of myelin-like structures, which appeared in the examined micrographs, it could be an indication of the insecticide induced stress (Singh and Singh, 1984; Sorour and Larink, 2000). It is known that neurons under stress accumulate membranous bodies because the cellular organelles undergo destruction and digestion while the undigested material accumulates as residual bodies (Essawy, 2009).

The present results indicate that treatment with DMDS induced degenerated synaptic vesicles, swelling of mitochondria and dilatation of their cristae in the axons of the neuropile. Similar observations were described by Tsunoda *et al.* (2006) and El-Sayed (2010). Degradation of the synaptic

vesicles could be attributed to the interruption of axonal transport which may promote degradation of synaptic terminals. Abundance of mitochondria and their accumulation were quite evident in the cytoplasm of the neuroglia cells after treated the cockroaches with DMDS. Such observations have been observed in various tissues subject to various pathological stages where an increased functions demand is made on the tissue (Ghadially, 1982). However, mitochondrial swelling reflects deregulation of mitochondrial membrane transport (Braeckman *et al.*, 1999). Dugravot *et al.* (2003) reported that dimethyl disulfide exerts an uncommon complex mode of action through mitochondrial dysfunction.

From the previously discussed results, it could be concluded that DMDS has a highly neurodegenerative effect and could be used in pest control.

Corresponding Author: Prof. Dr. Amina, E. Essawy, Zoology Dep. Faculty of Science, Alexandria University, Alexandria, Egypt.
E-mail: amina_essawy@yahoo.com

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