Effect of vitamin E supplementation on dichlorvos-induced toxicity in the hippocampus of male albino rat: a light-microscopic study

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Background
The hippocampus is a brain structure that plays important roles in the acquisition of new memories. Dichlorvos or 2,2-dichlorovinyl dimethyl phosphate (DDVP) is an organophosphate compound that is toxic to the hippocampus. Vitamin E is an antioxidant.

Aim
To evaluate the potential ameliorating effect of vitamin E supplementation on dichlorvos (DDVP)-induced toxicity in the hippocampus of male albino rat.

Materials and methods
In total, 40 male-adult rats aged 3 months were divided into five groups: group A (control): they received no treatment. Group B (treated): DDVP given at a dose of 2.5 mg/kg body weight/day for 4 weeks. Group C (protective): DDVP at the same previous dose cotreated with vitamin E at a dose of 200 mg/kg body weight/day for 4 weeks. Group D (therapeutic): DDVP given at the same previous dose for 4 weeks. Post-DDVP administration, rats were further treated with vitamin E at the same previous dose for another 4 weeks. Group E (rehabilitated): DDVP given at the same previous dose for 4 weeks. Post-DDVP administration, rats were further treated with distilled water for another 4 weeks. For each group, brains of rats were processed for light microscopy (gallocyanin chrom alum stain).

Results
Group B (treated) showed degenerative changes in the hippocampal principal cells. Group C (protective), showed improvement of cells more or less similar to control. Group D (therapeutic) showed improvement of cells near to control. Group E (rehabilitation) showed marked deterioration of cells in comparison with control.

Conclusions
DDVP had a toxic effect on rat hippocampus, that did not improve by rehabilitation. Vitamin E was found to be necessary for amelioration of the hazards of DDVP toxicity and its protective effect is more obvious than its therapeutic effect.

Keywords:
dichlorvos, hippocampus, vitamin E

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Introduction

The hippocampus is a major component of the brain [1]. It plays important roles in the acquisition of new memories and is intimately involved in learning and spatial cognition [2].

Dichlorvos or 2,2-dichlorovinyl dimethyl phosphate (DDVP) is an organophosphate compound [3]. It is widely used as an agricultural insecticide on crops and domestic animals [4]. In the form of pesticide-impregnated plastic, it is used in pet collars for dogs [5]. Dichlorvos is highly toxic by inhalation, dermal absorption, and ingestion; meanwhile, it may cross the placenta [6].

Vitamin E is an antioxidant that can cross blood–brain barrier [7].

Materials and methods

The experiments were performed at the Human Anatomy and Embryology Department of Assiut University, Faculty of Medicine.

Drugs used in this experiment, dichlorvos: 2.5 mg/kg/day dissolved in corn oil and was given subcutaneously. This dose was chosen because it provoked oxidative stress without lethal effects in rats [8]. Vitamin E: 200 mg/kg/day was given orally [9]. Forty male-adult rats aged 3 months were divided into five groups: group A (control) (eight rats): they received

no treatment. Group B (treated) (eight rats): DDVP was given daily for 4 weeks. Group C (protective) (eight rats): DDVP cotreated with vitamin E daily for 4 weeks. Group D (therapeutic) (eight rats): DDVP given daily for 4 weeks. Post-DDVP administration, rats were further treated with vitamin E for another 4 weeks. Group E (rehabilitated) (eight rats): DDVP given daily for 4 weeks. Post-DDVP administration, rats were further treated with distilled water for another 4 weeks.

Animals were anesthetized with diethyl ether inhalation, subjected to intracardiac perfusion with normal saline 0.9% NaCl, and then sacrificed. Brains of rats for each group were extracted and were processed for light microscopy (gallocyanin chrom alum stain). The research was reviewed and approved by the committee of medical ethics of the Faculty of Medicine, Assiut University on 30 of October 2016 and the approval IRB was 17101341.

Results

Light-microscopic examination (gallocyanin stain)

Dentate gyrus

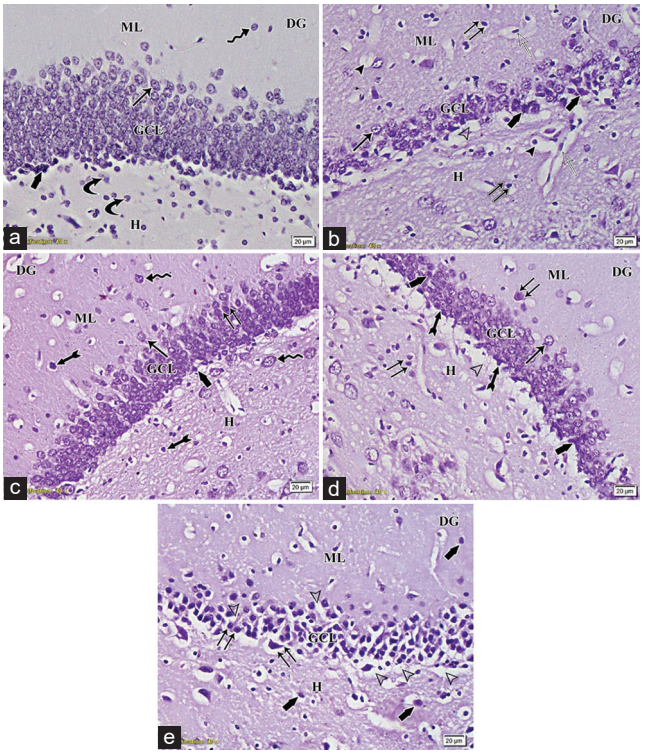
Examination of dentate gyrus of the control group demonstrated that the principal layer, granular cell layer composed of granule cells arranged in —five to six rows of cells. The granule cells had rounded vesicular nuclei with prominent nucleoli (Fig. 1a). The granular cell layer in the DDVP-treated group appeared with less rows comparable to control and invaded with dark-shrunken cells and vacuolations mostly in deep rows and in the subgranular zone. Some cells with vesicular nuclei were seen in the most superficial rows (Fig. 1b).

In DDVP + vitamin E-protected group, the granular cell layer retained its integrity as control and the granular cells were regularly arranged and appeared with rounded vesicular nuclei. Few cells with pyknotic nuclei were also detected (Fig. 1c). In vitamin E after DDVP therapeutic group, the granular layer appeared reduced with less rows compared with control. The granular layer still exhibited some dark cells and vacuoles (Fig. 1d). The rehabilitated group showed more or less loss of normal architecture of the granular layer that showed shrunken cells with pyknotic nuclei. Vacuolations and dispersions between its cells were also detected (Fig. 1e).

CA1 region

Examination of the control group showed that the principal cell layer, stratum pyramidale, was

Figure 1



(a) A photomicrograph of dentate gyrus (DG) in adult control rat. It shows the granular cell layer (GCL) composed of cells with rounded vesicular nuclei arranged in several rows. The superficial layers show larger vesicular cells (arrow), while small dark cells (thick arrow) are seen in the subgranular zone. The molecular layer (ML) has small sporadic distributed vesicular nuclei (wavy arrow). The polymorphic layer or hilus (H) shows cells of different shapes and sizes (curved arrow). Gallocyanin, ×400. (b) A higher magnification of a coronal section in the dentate gyrus (DG) of an adult DDVP-treated rat showing granular cell layer (GCL) appears with dark-stained nuclei (thick arrow), while few cells still have rounded vesicular nuclei (arrow). There is obvious vacuolation (open arrow head) in the subgranular zone. Note also that the molecular layer (ML) and the polymorphic layer or hilus (H) have degenerated cells with pyknotic nuclei (double arrow), vacuolations (arrow head), and dilated blood capillaries (open arrow). Gallocyanin, ×400. (c) A photomicrograph of a coronal section in the dentate gyrus (DG) of DDVP + vitamin E-protected group showing that most cells in the granular cell layer (GCL) have round vesicular nuclei (arrow) more or less similar to the control. Few cells with pyknotic nuclei (double arrow) are also found in this layer. The subgranular zone appears with small darkly stained cells (thick arrow) as control. Cells in the polymorphic layer or hilus (H) and molecular layer (ML) appear normal with vesicular nuclei (wavy arrow). Note also the presence of some darkly stained nuclei (tailed arrow) in these two layers. Gallocyanin, ×400. (d) A photomicrograph of a coronal section in the dentate gyrus (DG) of vitamin E after DDVP therapeutic subgroup showing that some cells of the granular cell layer (GCL) are still shrunken with darkly stained nuclei (thick arrow). While other cells appear normal with round vesicular nuclei (arrow) in this layer. The subgranular zone shows vacuolations (open arrow head) and small dark cells (tailed arrow). Note the presence of pyknotic nuclei (double arrow) in the molecular layer (ML) and polymorphic layer or hilus (H). Gallocyanin, ×400. (e) A photomicrograph of a coronal section in the dentate gyrus (DG) of an adult-rehabilitated rat showing that nearly all cells in the granular cell layer (GCL) are shrunken with pyknotic nuclei (double arrow). There is obvious vacuolation (open arrow head) in this layer and its subgranular zone. Note also that the molecular layer (ML) and the polymorphic layer or hilus (H) have shrunken cells with darkly stained nuclei surrounded by vacuolations (thick arrow). Gallocyanin, ×40. DDVP, 2,2-dichlorovinyl dimethyl phosphate.

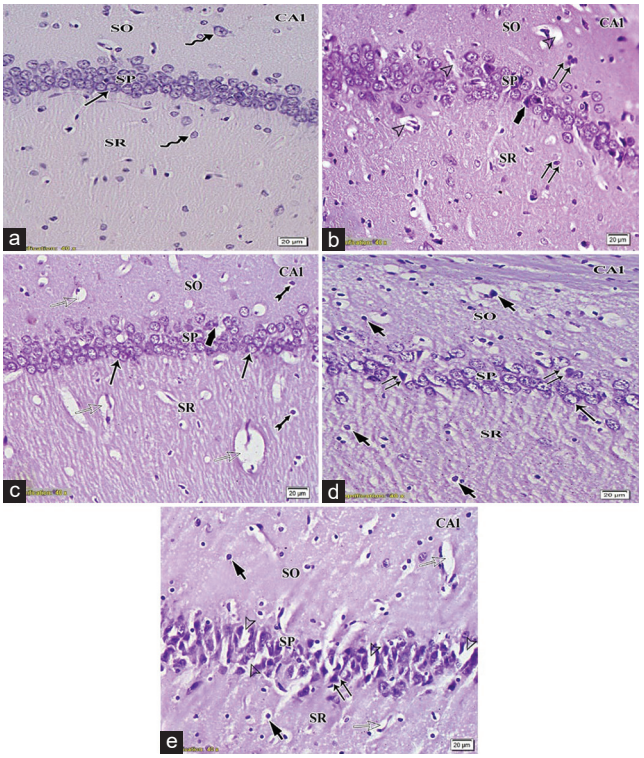
characterized by elongated pyramidal cells with a large ovoid to rounded vesicular nuclei and prominent nucleoli (Fig. 2a). The pyramidal cell layer in DDVP-exposed group appeared with reduced cellularity as compared with control and exhibited vacuolations and darkly stained cells (Fig. 2b).

DDVP + vitamin E-protected group showed a fairly good CA1 structure compared with those of control. The pyramidal neurons exhibited a large ovoid to round vesicular nuclei and prominent nucleoli. Few cells with darkly stained nuclei were also detected in this layer (Fig. 2c). Vitamin E after DDVP therapeutic group revealed few cells with pyknotic nuclei scattered in the pyramidal cell layer with cavitations (Fig. 2d). The pyramidal cell layer of the rehabilitated group displayed marked interruptions between the cells. It was found to be mostly composed of irregularly deeply stained cells and pyknotic nuclei (Fig. 2e).

CA3 region

Examination of CA3 field in the control group demonstrated that the principal cell layer (stratum pyramidale) was composed mainly of pyramidal neurons with large round vesicular nuclei and prominent nucleoli (Fig. 3a). The pyramidal cell layer in the DDVP group displayed deeply stained cells with pyknotic nuclei, surrounded by haloes and some neurons showed cytolysis or nucleolysis, other neurons appeared hazy-stained (Fig. 3b). DDVP + vitamin E-protective group retained the integrity of CA3 pyramidal cell layer and its neurons, which appeared with vesicular nuclei comparable to control. Few dark cells were detected in this layer (Fig. 3c). In vitamin E after DDVP therapeutic group, the pyramidal cell layer showed some neurons that appeared as cell ghosts, beside the normal vesicular neurons (Fig. 3d). While the pyramidal cell layer of the rehabilitated group exhibited marked disruptions between the pyramidal neurons that appeared with degenerate angulate nuclei (Fig. 3e).

Figure 2



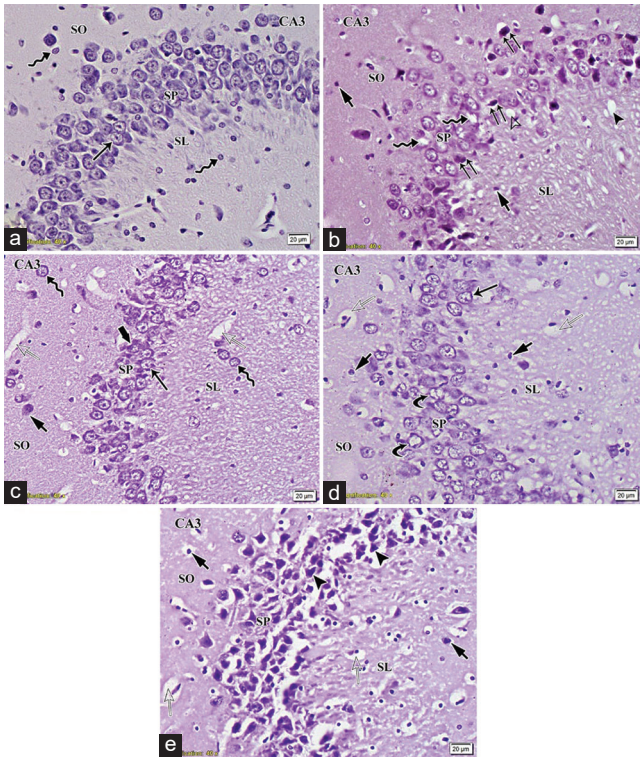
(a) A higher magnification of (CA1) region of the hippocampus in adult control rat showing that stratum pyramidale (SP) consists of regularly arranged pyramidal cells with large round-to-oval vesicular nuclei and prominent nucleoli (arrow). Some dispersed oval-to-round vesicular small nuclei (wavy arrow) are distributed in stratum oriens (SO) and in stratum radiatum (SR).Gallocyanin, ×400. (b) Higher magnification of (CA1) region in adult DDVP-treated rat shows disarrangement of the cells in stratum pyramidale (SP) as compared with control and some cells appear shrunken with darkly stained nuclei (thick arrow). Cells of stratum oriens (SO) and stratum radiatum (SR) are scanty with pykontic nuclei (double arrow). Some vacuolations (open arrow head) are seen in different layers. Gallocyanin, ×400. (c) Higher magnification of (CA1) region in DDVP + vitamin E-protected group showing that most pyramidal cells of stratum pyramidale (SP) appear to have large oval vesicular nuclei (arrow) more or less similar to that of the control. Few cells with darkly stained nuclei (thick arrow) are also found there. Note the presence of dark degenerated nuclei (tailed arrow) and dilated capillaries (open arrow) in stratum oriens (SO) and stratum radiatum (SR). Gallocyanin, ×400. (d) Higher magnification of (CA1) region in vitamin E after DDVP therapeutic subgroup showing that stratum pyramidale (SP) still has some degenerated cells with pyknotic nuclei surrounded by cavitation (double arrow). Other cells with vesicular nuclei (arrow) are also noticed in this layer, more or less similar to the control. Note the presence of cells with pyknotic nuclei (short arrow) in stratum oriens (SO) and stratum radiatum (SR). Gallocyanin, ×400. (e) Higher magnification of (CA1) region in adult-rehabilitated rat showing that stratum pyramidale (SP) appear with irregular deeply stained cells and pyknotic nuclei (double arrow). Note the marked disruption and vacuolation (open arrow head) in the surrounding neuropil. The stratum oriens (SO) and stratum radiatum (SR) appear with small degenerated cells with pyknotic nuclei (short arrow) and dilated capillaries (open arrow). Gallocyanin, ×400. DDVP, 2,2-dichlorovinyl dimethyl phosphate.

Discussion

In this work, DDVP (dichlorvos) was studied because it is one of the organophosphate compounds that is commonly used as an agricultural insecticide on crops and domestic animals in developing countries [3]. So, occupational exposure to dichlorvos is very common in farmers [6]. On entering the body, DDVP has its detrimental effects on lung and liver [10], kidney [11], as well as on the reproductive system [12].

The light-microscopic examination of the DDVP-treated group showed that there were obvious degenerative changes in the principal cells in the form of darkly stained, degenerated angulated nuclei with swollen vacuolated cytoplasm. These results were in accordance with Owoeye *et al.* [13] who studied the toxic effects of dichlorvos on the microanatomy of rat

Figure 3



(a) A higher magnification of (CA3) region in the adult control rat showing that the stratum pyramidale (SP) is mostly composed of pyramidal cells with large rounded vesicular nuclei, prominent nucleoli, fine distributed chromatin, and basophilic cytoplasm (arrow). Some scattered small vesicular cells (wavy arrow) are detected in stratum oriens (SO) and stratum lucidum (SL). Gallocyanin, ×400. (b) Higher magnification of (CA3) region in adult DDVP-treated rat showing that stratum pyramidale (SP) have deeply stained cells with pyknotic nuclei surrounded by haloes (double arrow). Some cells appear with cytolysis and nucleolysis (wavy arrow). Some vacuolations are also seen in this layer (open arrow head). Note that small deeply stained nuclei (short arrow) and vacuolations (arrow head) are seen in stratum oriens (SO) and stratum lucidum (SL). Gallocyanin, ×400. (c) Higher magnification of (CA3) region in DDVP + vitamin E-protected group. Most cells of stratum pyramidale (SP) have large round vesicular nuclei with prominent nucleoli (arrow) more or less similar to the control. Few small dark cells (thick arrow) are also detected in this layer. Vesicular nuclei (wavy arrow) with some small deeply stained nuclei (short arrow) and some dilated capillaries (open arrow) are seen distributed in other layers of CA3. SO (stratum oriens), SL (stratum lucidum). Gallocyanin, ×400. (d) Higher magnification of (CA3) region in vitamin E after DDVP therapeutic subgroup showing that stratum pyramidale (SP) has some faintly stained cell ghost and vacuolated cells (curved arrow). Other cells with large round vesicular nuclei (arrow) more or less similar to the control are also detected in this layer. Note the presence of deeply stained nuclei (short arrow) and dilated capillaries (open arrow) in stratum oriens (SO) and stratum lucidum (SL). Gallocyanin, ×400. (e) Higher magnification of (CA3) region in adult-rehabilitated rat. Nearly all pyramidal cells in stratum pyramidale (SP) have degenerate angulated nuclei with vacuolation (arrow head) in intervening neuropil. Note also cells with deeply stained nuclei (short arrow) and dilated capillaries (open arrow) in other layers. SO (stratum oriens), SL (stratum lucidum). Gallocyanin, ×400. DDVP, 2,2-dichlorovinyl dimethyl phosphate.

hippocampal formation. DDVP exerts its toxic effect by irreversibly inhibiting neural acetylcholinesterase. The inhibition provokes the accumulation of acetylcholine in synapses with disruption of nerve function [14].

In this work, giving vitamin E with DDVP simultaneously (protective) kept the normal architecture of the hippocampal fields. Most of the granule and pyramidal cells of the three regions appeared normal with vesicular nuclei, this came in agreement with Owoeye *et al*. [13]. When vitamin E was given in this study after DDVP exposure (therapeutic group), the light-microscopic examination revealed improvement of some cells that appeared with vesicular nuclei, while other cells had pyknotic darkly stained nuclei. These results were more or less similar to the results of Owoeye *et al*. [13].

Vitamin E as an antioxidant acts as a major free radical chain terminator. It might have terminated such peroxidative reaction due to exposure to DDVP, so vitamin E helps to ameliorate the neurotoxic effect of DDVP in the brain [15].

On light-microscopic examination of the rehabilitated group, nearly all principal cells of the three hippocampal regions were shrunken with darkly stained or pyknotic nuclei or degenerated as compared with control rats. These results were more or less similar to the results of Ojo *et al*. [16] who studied DDVP-exposed group in their studies of dichlorvos-induced oxidative stress in rat brain. It is concluded that DDVP had a toxic effect on rat hippocampus, that did not improve by rehabilitation. Vitamin E was found to be necessary for ameliorating the hazards of DDVP toxicity and its protective effect is more obvious than its therapeutic effect.

Inhibited marked disruptions between the pyramidal neurons appeared with degenerate angulated nuclei (Fig. 3e).

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Conflicts of interest
There are no conflicts of interest.

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