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The effect of vitamin supplementation on the toxic effects of dichlorvos on the microanatomy of rat hippocampal formation

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ABSTRACT

Dichlorvos (DDVP) is a widely used pesticide that is toxic to animals and humans but study of its effect on the microanatomy of the brain is scanty. This study was designed to investigate the ameliorating effect of vitamin supplementation on DDVP-induced neurotoxicity in the hippocampus of Wistar rats. 25 male Wistar rats were separated into unexposed group and those exposed to DDVP (1000 mg/L) through inhalation either without, or with vitamin E, vitamin C or red palm oil supplementation. Treatment lasted for 14 days after which rats were sacrificed by ketamine anaesthesia. Hippocampal biopsies were processed into paraffin blocks and H&E stained sections were evaluated by light microscopy. DDVP administration elicited toxicity in the dentate gyrus, cornuammonis1 (CA1) and cornuammonis 3 (CA3) regions. There was pyknosis and alteration of the microanatomy of dentate granule cells and pyramidal cells of CA1 and CA3. DDVP-induced toxicity was mitigated by vitamins E, C and red palm oil in the dentate gyrus, but partially in CA1 and CA3. Inhalational DDVP induces toxicity in the hippocampus of rats and this could affect memory. Toxicity of DDVP is partially ameliorated by vitamins E, C and red palm oil.

Keywords: Dichlorvos, dentate gyrus, cornuammonis, hippocampus, red palm oil.

INTRODUCTION

Memory is an important component of cognition and it must first be encoded by the hippocampal formation before the memory trace is transferred to other parts of the brain for short and long-term storage (Tranel, 1995; Hendelman, 2000). The hippocampus is a brain area that is crucial for the acquisition of new memories, and is intimately involved in learning and spatial cognition (Somogyi, 2010; Vivar and van Praag, 2013). Three hippocampal subfields: dentate gyrus (DG), area CA3 and area CA1 (Kesner, 2007) each have specific cell types and plasticity contributing to learning and memory processes (Nakazawa et al., 2004). Neural information is sequentially processed from the entorhinal cortex (EC) to DG, CA3, CA1 and subiculum, to be ultimately stored in EC, forming the intrinsic hippocampal circuit

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(Amaral and Witter, 1989). Damage to any of these hippocampal structures will affect encoding of memory which will adversely affect the quality of life.

According to the World Health Organization's estimate, 3 million cases of pesticide poisoning occur every year, resulting in more than 250,000 deaths (Binukumar and Gill, 2010). Chronic exposure to pesticides could result in tissue accumulation and cause tissue injury. Dichlorvos DDVP (O-O-dimethyl-O-2, 2-dichloro-vinyl phosphate) (USEPA, 2007), is an organophosphorous compound largely used to control ectoparasites in livestock and humans (Luty et al., 1998; Sharma and Singh, 2010). Organophosphorus compounds are primarily recognized to be neurotoxic in mammals as they inhibit the activity of acetylcholinestrase (AChE), an enzyme that decomposes acetylcholine (Hazarika et al., 2003). The inhibition of AChE results in an accumulation of excessive amounts of acetylcholine in synaptic clefts and muscular motor plates, thereby producing both nicotinic and muscarinic signs and symptoms of endogenous poisoning and intoxication in the peripheral and central nervous systems (Akinyoola et al., 2012). In Nigeria, a popular pesticide traded under the names such as "*Ota piapia*" or "*Nuvan*" has been reported to contain DDVP as the preponderant active pesticide ingredient (Musa et al., (2010). DDVP toxicity has been reported in reproductive system (Joshi et al., 2003), pancreas (Hagar et al., 2002), kidney and spleen (Verma and Srivastva, 2003), immune system (Neishabouri et al., 2004), liver and lungs (Owoeye et al., 2012); on haematological indices (Edem et al., 2012) and nervous tissue (Luty et al., 1998; Sharma and Singh, 2012). Studies support a role for reactive oxygen species (ROS) in the mechanism of DDVP toxicity (Sharma and Singh, 2012) and excessive generation of ROS causes irreversible impairment of DNA and damage to membrane lipids during the production of malondialdehyde (Arinola et al., 2011). Pesticides are believed to damage the lipoidal matrix in cells, generating reactive oxygen species (ROS) and promoting oxidative stress. Dueto continuous exposure of pesticides, the level of these antioxidants decreases thus leading to accelerated cell death (Sharma and Singh, 2012).

Exogenous antioxidants capable of neutralizing the effects of ROS include ascorbic acid (vitamin C, VTC), alpha-tocopherol (vitamin E, VTE) and red palm oil (RPO) among others. VTC isan electron donor, aproperty that accounts for all its known functions including its being a potent water-soluble antioxidant in humans, an effect that has been demonstrated in many experiments (Padayatty et al., 2003). VTE is an intracellular compound associated with lipid-rich biological membranes including those of mitochondria and endoplasmic reticulum; it is lipophilic making it a major free radical chain terminator (Singh, 2002). RPO is an edible vegetable oil derived from the mesocarp of the fruit of the African oil palm *Elaeisguineensis.* It is naturally reddish in colour because of a high content of carotenes, such as alpha-carotene, beta-carotene (contains vitamin A), lycopene, and at least 10 other carotenes, along with tocopherols and tocotrienols, phytosterols, and glycolipids. RPO is thus rich in both vitamin E and A (Bonnie and Choo, 2000).

In previous studies of the morphological changes in nervous tissue, DDVP was administered orally or dermally (Luty et al., 1998; Desi and Nagymajtenyi, 1998). It is important to evaluate the effects via inhalation. This is so because the general population is more likely to be exposed to pesticides through inhalation of contaminated indoor air during and/or immediately after application of pesticides like DDVP as insecticides. DDVP generates reactive oxygen species which damage membrane lipids and since the brain is rich in lipids, it is hypothesised that inhaled DDVP may have impact on the brain. The objective of this research was to investigate the possible effect of inhalational DDVP on the microscopic structure of the hippocampus of rats and so test the hypothesis that antioxidant-containing vitamins could protect the neurons from

DDVP-induced toxicity. This should assist us to answer the research question of whether vitamin supplementation can modulate the histological alteration elicited by inhalational DDVP-induced toxicity in brain of Wistar rats.

MATERIALS AND METHODS

Experimental animals and materials

This study was carried out using 25 ten weeks old Wistar male rats (75.05 ± 5.55 g) obtained from the Animal Holding facilities of Anatomy Department, University of Ibadan, Nigeria. They were acclimatized to laboratory room conditions (12 hours dark – light period) for 2 weeks before the onset of treatment. The rats were fed during the acclimatization period with rat chow from Ladokun Feeds, Ibadan, and water *ad libitum.* Vitamins E and C were purchased from Dana Pharmacy, Ibadan, Nigeria, while RPO was purchased from Bodija market, Ibadan. DDVP was purchased from Farmers Shopping Plaza, Ogunpa, Ibadan, Nigeria. The experimental protocols were carried out according to acceptable guidelines on the ethical use of animals in research (Public Health Service, 1996).

Research design

After the period of acclimatization, the 25 rats were randomized into 5 equal groups as follows using a modification of the method of Edem et al. (2012). Group I: control (unexposed); Group II: exposure to DDVP alone for 4 weeks; Group III: exposure to DDVP for 4 weeks plus vitamin E (1.25 g/kg feed per day); Group IV: exposure to DDVP for 4 weeks plus vitamin C (106 mg/kg body weight per oram per day); Group V: exposure to DDVP for 4 weeks plus RPO (2 ml/25g feed per day). The dosage of vitamin E was based on the method of Murthy et al. (1992), while that of vitamin C was according to the method of Ambali et al. (2011). DDVP (1000 mg/L) was prepared freshly daily in a dilution of 1:1 as recommended by the manufacturer (Hubei Samonda Co. Ltd, China), i.e. 50 mL of DDVP was mixed with 50 mL of clean water. The groups were separated in different cages. To simulate a poorly ventilated compartment, rats were placed in Perspex cages (35.5 cm x 27.5 cm x 19 cm), the DDVP preparation was placed beside the cage and each cage covered with a cardboard carton with only one of the upper two lids partially opened. Rat exposure to DDVP in all 5 groups lasted 4 hours daily by this inhalational method after which the DDVP solution container and the carton were removed.

Sample collection and histological preparation

At completion of exposure, the animals were anaesthetized with Ketamine (10 mg/kg intraperitoneally). Thereafter, the brain of each animal was removed and fixed in 10% formalin for 7 days. The brains were then sectioned coronally and biopsies were obtained from the hippocampal formation at the level of the lateral geniculate body. These biopsies were subsequently processed by dehydration with increasing grades of ethanol (70, 80, 90, 95 and 100%). Dehydration was then followed by clearing the samples in 2 changes of xylene followed by impregnation with 2 changes of molten paraffin wax, then embedded and blocked out. Paraffin sections (5-6µm) thick transverse sections of the hippocampal formation were cut using a rotary microtome (Leica RM2125RTS, Germany), and then mounted on glass slides. The slides were stained with haematoxylin and eosin (H&E) according to the method of Bancroft and Gamble (2008).

Histology

Stained sections were examined with the light microscope (Olympus CH Japan) for histopathological studies. Special attention was paid to the dentate gyrus, CA1 and CA3 regions. Photomicrographs of these 3 regions were taken from each animal with a Sony DSC-W 30 Cyber-shot (Japan) camera at x 240 magnification. A scale bar was inserted in the photomicrograph using Image J (Schneider et al., 2012).

RESULTS

General

Our observations showed that the neurons of control animals in the DG, CA 1, and CA 3 parts of the hippocampal formation exhibited large soma with copious cytoplasm. The nuclei of the neurons are large, ovoid or round, chromatin was dispersed and the nucleoli were conspicuous and prominent as shown in Figures 2A, 4A, and 6A.

Histopathology of DG

The DG of rats exposed to DDVP showed scattered pyknotic neurons, reduced cellularity as well as shrunken size as shown in Figure 2B. The brain of rats that received supplementation of VTE, VTC and RPO showed fairly good structure as shown by their neurons comparing well with those of control rats as displayed in Figures 3C, 3D, and 3E.

Histopathology of CA1

Rats exposed to DDVP exhibited scattered degenerate and pyknotic neurons and reduced neuronal density in CA1 as shown in Figure 4B. Rats that received supplementation of VTE and VTC showed CA1 structure comparable with exposed groups as shown by few pyknotic cells and degenerate angulated neurons (Figure 5C and 5D). Rats that were treated with RPO supplementation showed fewer pyknotic cells and milder hypocellularity as compared with those that received VTE and VTC (Figure 5E).

Histopathology of CA 3

Rats exposed to DDVP showed patchy areas of neuronal loss, shrunken and degenerate angulated neurons in CA3 as displayed in Figure 6B. Rats that received supplementation of VTE and VTC showed reduction of cellular densities, few pyknotic cells and degenerate neurons as shown in Figures 7C and 7D. Rats that received RPO supplementation showed better preservation of CA3 neurons compared with the VTE and VTC as shown in Figure 7E which is comparable with the control of Figure 6A.

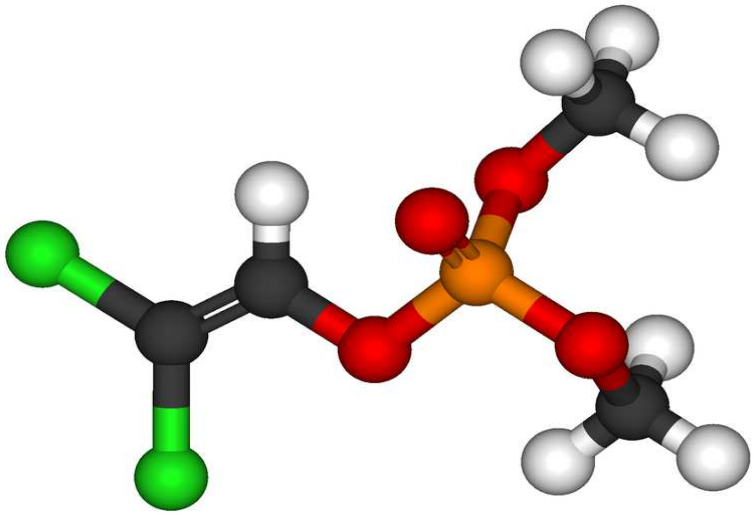

Figure 1: The ball-and-stick model of dichlorvos (O.-Odimethyl-O-2, 2-dichloro-vinyl phosphate) (USEPA, 2007).

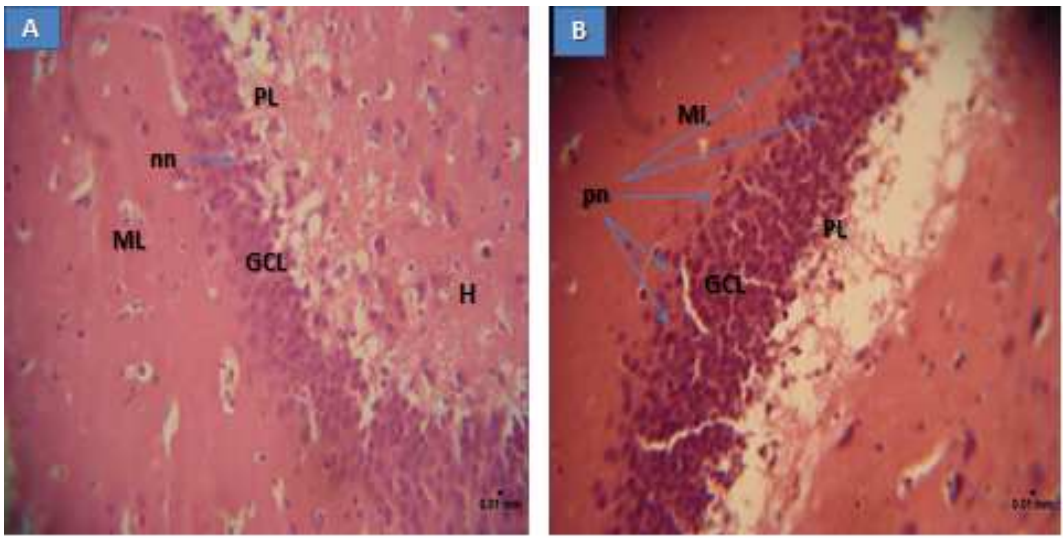

Figure 2: Representative photomicrographs of dentate gyrus of hippocampus of rats. A: control; B: DDVP exposure for 4 weeks; H &E: stain; nn: normal neuron; pn: pyknotic neuron; ML: molecular layer; GL: granular layer; PL: polymorphic layer; H: hilus; Pyknotic granule cells are observed in B; Scale bars indicate 0.01 mm (10 µm).

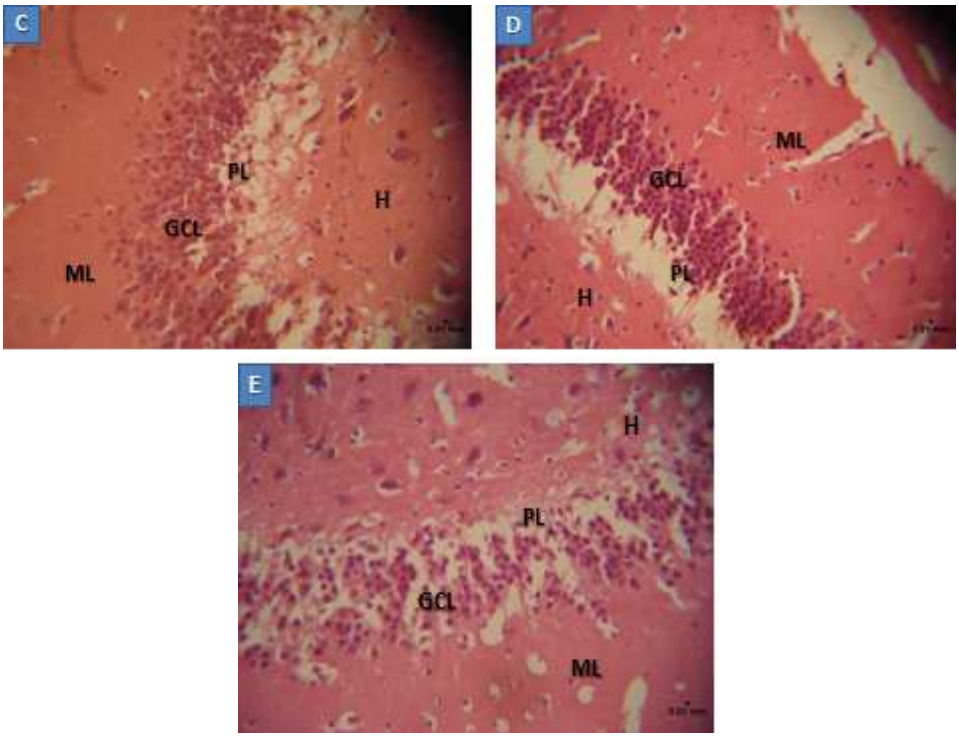

Figure 3: Representative photomicrographs of dentate gyrus of hippocampal formation of rats. C: DDVP+VTE; D: DDVP+VTC; E: DDVP + RPO; H &E: stain; nn: normal neuron; pn: pyknotic neuron; ML: molecular layer; GL: granular layer; PL: polymorphic layer; H: hilus; VTE: vitamin E; VTC: vitamin C; RPO: red palm oil; Pyknotic neurons are not observed in A, B and C. Scale bars indicate 0.01 mm (10 µm).

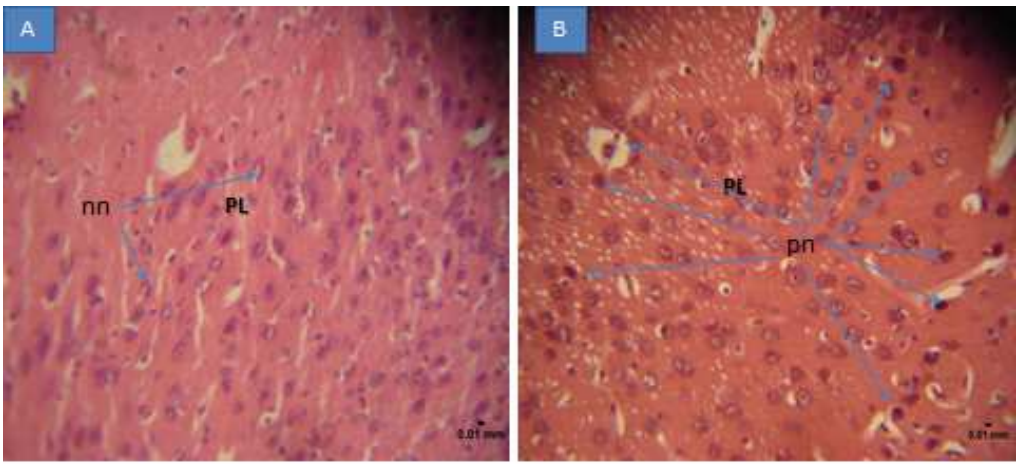

Figure 4 : Representative photomicrographs of cornuammonis 1 field of hippocampus of rats. A: control; B: DDVP exposure for 4 weeks; H &E: stain; nn: normal neuron; pn: pyknotic neuron; PL: pyramidal layer containing pyramidal neurons. Scaterred pyknotic pyramidal neurons are observed in B; Scale bars indicate 0.01 mm (10 µm).

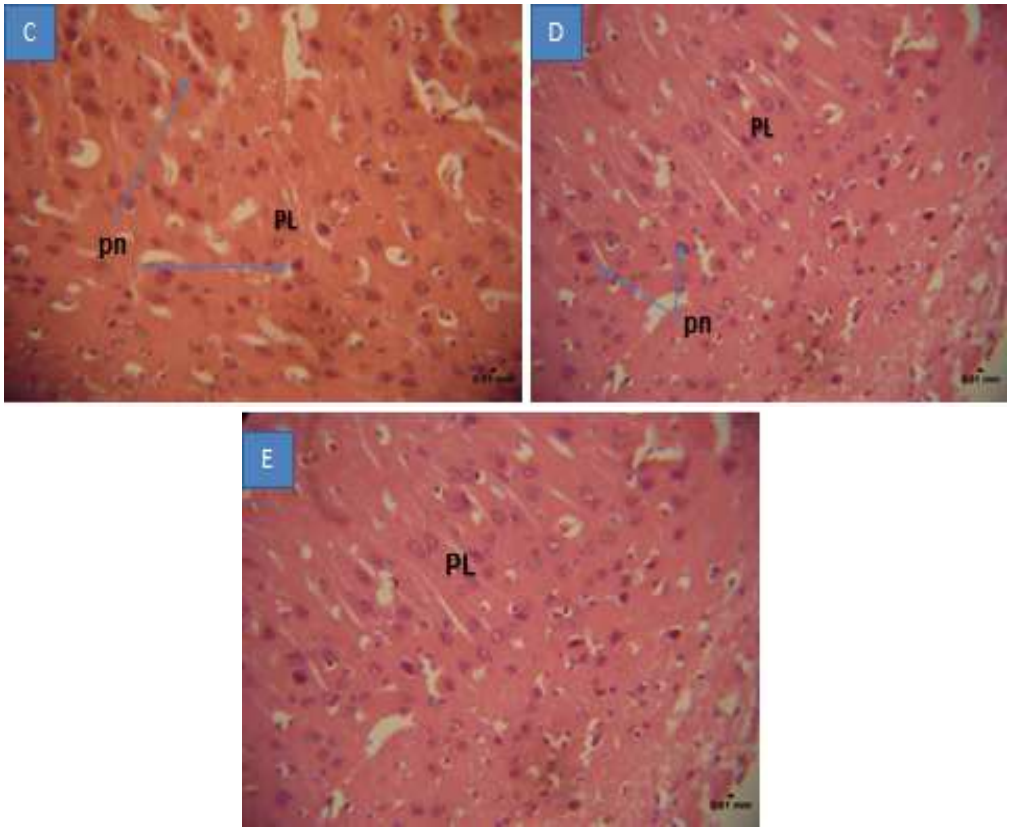

Figure 5 : Representative photomicrographs of cornuammonis 1 field of hippocampus of rats. C: DDVP+VTE; D: DDVP+VTC; E: DDVP + RPO; H &E: stain; nn: normal neuron; pn: pyknotic neuron; PL: polymorphic layer; H: hilus; VTE: vitamin E; VTC: vitamin C; RPO: red palm oil; Pyknotic pyramidal neurons scanty in C and D but absent in E. Scale bars indicate 0.01 mm (10 µm).

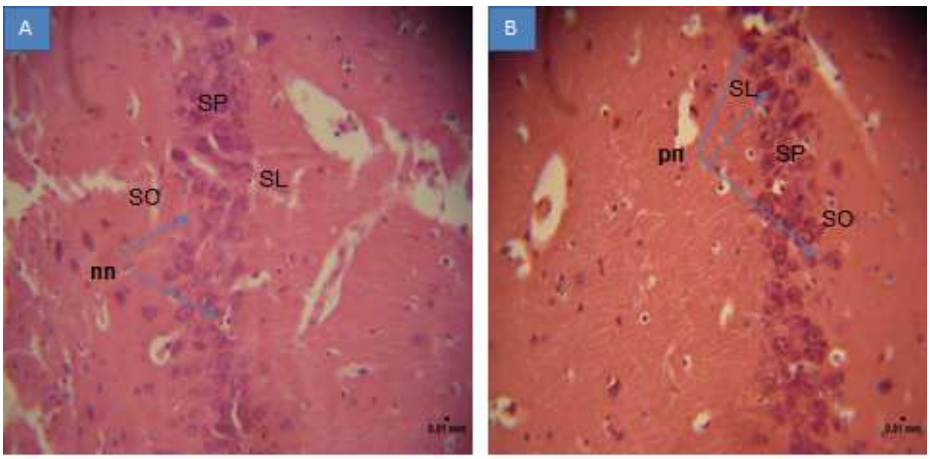

Figure 6 : Representative photomicrographs of cornuammonis 3 field of hippocampus of rats. A: control; B: DDVP exposure for 4 weeks; H &E: stain; nn: normal neuron; pn: pyknotic neuron; SO: stratum oriens; SP: stratum pyramidales; SL: stratum lucidum; Few scaterred pyknotic pyramidal neurons are observed in B; Scale bars indicate 0.01 mm (10 µm).

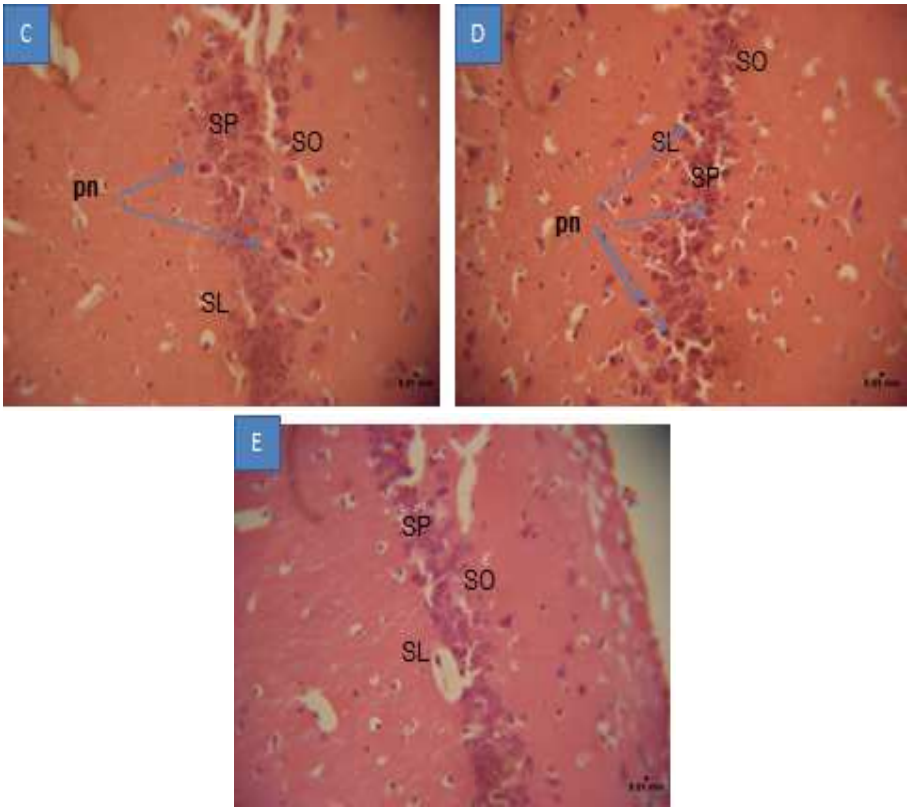

Figure 7 : Representative photomicrographs of cornuammonis 3 field of hippocampus of rats. D: DDVP+VTE; E: DDVP+VTC; F: DDVP + RPO; H &E: stain; nn: normal neuron; pn: pyknotic neuron; SO: stratum oriens; SP: stratum pyramidales; SL: stratum lucidum; VTE: vitamin E; VTC: vitamin C; RPO: red palm oil; Pyknotic pyramidal neurons scanty in C and D but absent in E. Scale bars indicate 0.01 mm (10 µm).

DISCUSSION

This study has shown the neurotoxic effects of DDVP on the hippocampal formation of rats exposed to chronic inhalation of DDVP and the effects of vitamins on DDVP neurotoxicity. Reduced cellularity of the dentate gyrus, with neuronal degeneration and pyknosis of the dentate granule cell (DGC) nuclei is indicative of the toxicity of DDVP. Similar changes were seen in the pyramidal neurons of the CA1 and CA3 subfields, which is in agreement with the findings of Luty et al. (1998) who reported similar pyknosis in the pyramidal neurons of CA1 of rats treated dermally with DDVP. This suggests that DDVP exhibited its neurotoxicity irrespective of the route of administration since our rats were exposed to inhalational DDVP.

The neurotoxicity of DDVP may be due to oxidative damage induced by DDVP as described by Sharma and Singh (2012). Severe oxidative damage may lead to cell death by necrosis exemplified by the presence of nuclear pyknosis. The brain is considered more vulnerable to oxidative stress than any other organ of the body because it consumes high amount of oxygen, contains high content of polyunsaturated fatty acids (PUFA) and low level of antioxidant enzymes (Sharma and Singh, 2012).

The hippocampus proper and the dentate gyrus, as part of the hippocampal formation of the limbic system, play important roles in the acquisition of new memories, and are intimately involved in learning and spatial cognition (Somogyi, 2010; Vivar and van Praag, 2013) as well as in explicit long-term recognition memory (Shapiro and Eichenbaum, 1999; Olsen et al., 2012). They are also involved in cognitive functions especially in the areas of relational binding involving the association of disparate elements and comparison of externally presented information with stored/associated memory representations (Aloisi et al., 1997; Olsen et al., 2012). Oxidative damage to the sub-ventricular zone of DG might affect the generation of new dentate granule cells (DGCs) as this would disturb the equilibrium of new neurons that should be added to the dentate gyrus (DG) of the adult mammalian brain (Piatti et al., 2013).

Pyknotic neurons indicate the onset of necrotic cell death (Stevens and Lowe, 2000) which is an indication of the toxicity of the chronic inhalation of DDVP, the implication of which is a potential reduction in the functional efficiency of the classical intrinsic hippocampal circuit neuronal pathways associated with these neural structures. In this circuit, information is considered to be processed from entorhinal cortex (EC) to DG, DG to CA3 pyramidal cells, and from CA3 to CA1 pyramidal cells to be ultimately stored in cerebral cortex (Amaral and Witter, 1989; Nakazawa et al., 2004). Functionally, neural information relays from the entorhinal cortex via two major cortical inputs, the medial entorhinal cortex (MEC) and lateral entorhinal cortex (LEC) (Johnston and Amaral, 1998; Van Cauter et al., 2012). This comes in via the perforant path to the molecular layer of DG and may be affected when there is neuronal death in terms of impulse transmission and relay (Ming and Song, 2011). DGCs are the principal neurons of the DG, which constitute the main gateway to the hippocampus (Piatti et al., 2013). Granule cell death might affect the passage of neural information coming in to the molecular layer of DG. The second part of this circuit arises from the projection cells of the dentate gyrus, the granule cells, which send their axons to the pyramidal cells of CA3 (Ribak and Shapiro, 2007. Neuronal death would no doubt affect the axons of DGCs (mossy fibres) arising from the granular layer of DG as they proceed on the way to CA3 (Jaffe and Gutiérrez, 2007). It is from CA3 that Schaffer's collaterals will project on to the CA1 neurons, and finally to the subiculum, en route to entorhinal cortex,

though CA3 may project back directly to the DG (Scharfman, 2007). Neuronal death along the pathway reduces the effectiveness of the neural information which may lead to a reduction in the function of the hippocampal formation in its memory encoding and long-term recognition memory capabilities.

Since ROS generation is the reported basis for the oxidative damage of DDVP (Sharma and Singh, 2012), the use of exogenous antioxidants should be able to neutralize the effects of ROS. As an electron donor, VTC donates an electron to free radicals to neutralize them as has been reported (Padayatty et al., 2003). Vitamin E (VTE) is an intracellular compound associated with lipid-rich biological membranes of which neuronal membranes made up of multilayers of lipid is a good example. Such membranes are subject to lipid peroxidation when exposed to the DDVP toxicity, but because of its lipophilic nature, VTE as a major free radical chain terminator might have terminated such peroxidative reaction (Singh, 2002). The combination of VTE in red palm oil in addition to beta-carotene and other type of carotenes enhances its anti-oxidative potentials, hence the amelioration observed in the granule cells of the brain of red palm oil-treated rats in our study. However, unlike the case with the fascia dentata where VTC and VTE effectively ameliorated the effect of DDVP-induced toxicity, both multivitamins did this partially in the CA1 and CA3 subfields of the hippocampus proper. Red palm oil, however, was able to reduce the effect of DDVP on the pyramidal neurons; this effect is most probably due to the combination of VTE in red palm oil in addition to vitamin A in form of beta-carotene and other type of carotenes (Bonnie and Choo, 2000). This must have increased the potency of the anti-oxidative activity of red palm oil helping it to ameliorate the neurotoxic effect of DDVP in the brain of red palm oil-treated rats.

Taken together, our findings imply that chronic inhalation of DDVP may adversely affect the memory component of cognition and that antioxidants as present in RPO may reduce this effect. It may also be inferred that patients suffering from degenerative neurological disorders like Alzheimer's disease should be prevented from undue exposure to pesticides containing DDVP since the basic pathology of their ailment is in the hippocampus and chemical injury of the hippocampal formation might tend to worsen such conditions. Younger healthy individuals should also be encouraged to take precautionary measures to avoid hippocampal injury due to undue and prolonged exposure to prevent impairment of learning and memory.

Conclusion

Inhalational DDVP caused toxic changes in the dentate granule neurons and pyramidal neurons of CA1 and CA3 of the hippocampus which were ameliorated by red palm oil and partially by vitamins E and C.

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