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ASCORBIC ACID MITIGATES DICHLORVOS- EVOKED HIPPOCAMPAL DEGENERATION IN MALE WISTAR RATS

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Abstract: Dichlorvos, a widely used pesticide, poses significant neurotoxic risks. This study examined the protective potentials of vitamin C on dichlorvos (DV)-induced hippocampal damage in Wistar rats. Five groups of rats were exposed to different aqueous dilutions of DV aerosol in a chamber for 4 hours daily over 21 days. Three groups were co-administered vitamin C (160 mg/kg) daily. Histological examination revealed that rats exposed to DV dilutions exhibited hippocampal damage characterized by pyknosis and structural alterations in the cornu ammonis and dentate gyrus. The Y-maze and novel object recognition tests revealed impaired short-term spatial (SM) and non-spatial memory (NSM). However, vitamin C supplementation ameliorated the extent of neurodegeneration in the hippocampus and the spatial and non-spatial cognitive deficit levels. The amelioration in NSM function was remarkable; there was no statistical difference between the control and vitamin C-supplemented groups. In contrast, there were significant differences between the supplemented groups and the rats exposed to DV without vitamin C supplementation. However, the ameliorative effect of vitamin C on SM impairment seems less pronounced; there was a statistical difference between the control and the supplemented groups and between the supplemented groups and the rats exposed to DV without supplementation. These findings highlight the neurodegenerative and apoptotic effects of dichlorvos on the hippocampus and suggest a potential benefit of vitamin C supplementation in mitigating the neurotoxic effects of DV.

Keywords: neurodegeneration, apoptosis, hippocampus, neuroprotection

1. Introduction

Dichlorvos is a widely used pesticide in less developed countries because of its potency and low cost (Nwankwo et al., 2019). It is a chemical classified (class 1B) by WHO as highly hazardous (WHO, 1992). It is an organophosphorous, colourless, aromatic compound. Furthermore, research indicates that undue exposure may cause damage to many organs, including the brain (Mostafalou and Abdollahi, 2017). The harmful effects of DV in most organs are linked to the production of free radicals, which impair mitochondrial function and cause inflammation and cell death. This cascade causes hepatocyte death and liver dysfunction (Saka et al., 2025). The consequences in the kidneys include renal tubular and glomerular necrosis, resulting in kidney damage (Adeoye et al., 2022). Reports

also suggested that this cascade triggers cardiomyocyte necrosis and cardiac impairment (Salem et al., 2023), a reduction in the mature spermatocyte population and vascular congestion in the testis (Saka et al., 2024). Neuronal damage coupled with neurological deficit have been reported in dichlorvos (DV) exposure (Imam et al., 2018). Certain in vivo studies have associated DV with oxidative stress, cognitive impairment, and hippocampal damage, leading to memory consolidation deficits (Farkhondeh et al., 2020; Anderson et al., 2023). Studies show that the hippocampus, a critical structure for memory formation, is particularly vulnerable to the neurotoxic effects of DV (Huang et al., 2022; Ommati et al., 2024). This is due to its high metabolic rate, a relatively weaker blood-brain barrier in its location, and its unique plasticity necessitated by its crucial role in learning and memory, which makes it susceptible to changes caused by various insults, including toxins like organophosphate (Davidson et al., 2024). According to Mostafalou and Abdollahi (2023), dichlorvos causes damage by blocking acetylcholinesterase activity in an irreversible manner, which leads to acetylcholine buildup in the synaptic cleft and excessive stimulation of cholinergic receptors. This can cause death, paralysis, or cognitive impairment (Saravanakumar et al., 2023).

Rajak et al. (2022) also demonstrated that DV induces neuroinflammation and oxidative stress, which damages hippocampal neurons. Consequently, this disruption of hippocampal function causes impairments in spatial and non-spatial recognition memory (Chauhan et al., 2021). Chronic exposure to DV has been implicated in neurodegenerative disorders and neuropathological lesions in the neocortex, hippocampus and cerebellum. These lesions were attributed to increased neuronal death (Yu et al., 2021; Sarailoo et al., 2022). Chronic DV exposure causes neuropathy affecting central and peripheral nervous system axons, potentially leading to paralysis (Uwaifo and John, 2020; Ranjan et al., 2022).

Apart from its agricultural use, it is also used as an aerosol spray for insect control in public institutions like schools, hospitals, and offices in developing counties (Clesceri et al., 2021; Mendoza et al., 2023).

Additionally, it is used as an anti-helminthic agent for pigs, horses, and dogs in veterinary practice and against crustacean ectoparasites that infect fish in aquaculture (Martins et al., 2021; Pattanayak et al., 2024). Recent studies, however, indicated that only a small proportion of the dichlorvos used reaches the targeted pests; over 90% go into soil and water as contaminants (Bankole et al., 2020; Kiruthiga, 2021), causing additional health risks. However, ascorbic acid, a natural antioxidant, has been shown to mitigate oxidative stress and improve cognitive function (Raeeszadeh et al., 2022). However, its potential protective effects against dichlorvos-induced toxicity on hippocampal structure and memory consolidation remain unclear. The primary endeavour of this study was to examine the protective effects of ascorbic acid against dichlorvos-induced toxicity on hippocampal structure and memory consolidation (**Fig. 1.**).

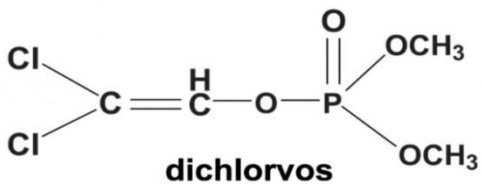

Fig. 1. Structural formula of Dichlorvos (Okoroiwu and Iwara, 2018)

2. Materials and methods

Animal acclimatization and handling

Twenty-five young adult (7 weeks old) male Wistar rats (180-200 g) were sourced from the pharmacology department's animal unit at Niger Delta University, Nigeria. We randomly assigned them to five groups (n = 5 per group) and housed them in a PVC cage (40 cm × 40 cm × 20 cm). Before beginning the experiment, they spent 21 days acclimating to standard laboratory conditions in the research vicinity, in 12 hours of daylight and 12 hours of darkness. Access to regular rat chew and water was unrestricted. All protocols in this study were approved by the College Ethics Committee, CHS, Niger Delta University (Ref No. 02-0852024/080).

Administration schedule

Dichlorvos (DV) concentration and administration followed previously established protocols (Ogunsola et al., 2019; Hart and David, 2022). The lethal inhalation concentration of DV was determined to be 50 ml per 50 ml of distilled water. Following these methods, the groups were treated as detailed (**Table 1**) for 21 days.

Table 1. Group and treatment regimen

Group (GP)	Amount DV in distilled water (v/v)	Vitamin C per oral supplementations
1	Nil	nil
2	40/60	160 mg/kg
3	10/90	160 mg/kg
4	20/80	160 mg/kg
5	40/60	160 mg/kg

Dichlorvos (DV) exposure

Rats were exposed to DV vapour in a partially ventilated PVC box. Each group was provided a box measuring 40 cm × 30 cm × 20 cm. Each box featured a perforated lid containing ten 2-cm-diameter ventilation apertures. Within each box was placed a cylinder (10 cm height × 10 cm diameter) containing cotton wool soaked with the appropriate DV solution for the particular group (**Table 1**). Groups 2-5 rats were introduced into the DV-inhalation boxes and were exposed to DV vapours emanating from this setup for four hours daily for 21 consecutive days.

Novel object recognition test (NORT)

Short-term non-spatial memory (NSM) was assessed through the novel object recognition as previously described (Lueptow, 2017). Briefly, a 40 cm × 40 cm transparent glass box was used for the novel object recognition test. This test is predicated on rodents' proclivity to naturally explore unfamiliar objects for longer periods than the accustomed ones. During the training phase, they were familiarised with the test box. Two identical objects were placed at equal distances within the arena, and each rat was allowed to explore for 5 minutes and then returned to the home cage. Thirty minutes later (retention interval), the rat was reintroduced and allowed to walk around the test arena with a familiar object (FO) and a new one (NO) for 5 minutes to test short-term non-spatial recognition memory. The objects and the test box were cleared with 70% alcohol after each test. We assessed cognitive deficit by analysing object

discrimination abilities during both the training (T1) and testing (T2) phases. The discrimination index (DI) was calculated using the formula (1):

$$DI = \left(\frac{Time\ spent\ with\ NO}{Time\ spent\ with\ NO + Time\ spent\ with\ FO}\right) 100 \ldots\ldots\ldots(1)$$

Where FO (familiar object) and NO (novel object)

This metric quantifies the preference for the novel object, providing an indicator of memory and discrimination ability.

Y-Maze test

This test was also to measure the short-term spatial memory of the animals by recording the number of triads and arm entries to calculate the proportion of alternation (Kraeuter et al., 2019). Using a wooden constructed three-arm Y-maze, 50 cm each, 12 cm wide, and standing 22 cm from a flat surface. Each animal spent five minutes exploring its various arms after being carefully positioned in the middle of the maze. By keeping track of the frequency of appropriate sequences between the arms, such as ABC, ACB, BCA, BAC, CBA, or CAB, the percentage of proper alternations was calculated. This test evaluates the animal's capacity for spatial memory and judgment. Analysis of correct alternation was estimated as previously described (Edem et al., 2022, formula 2). Briefly, the correct entries into all three arms without repetition, e.g., ABC, BCA, CAB percentage is:

$$\%Altern. = \left(\frac{Number\ of\ unique\ triads\ (e.g., ABC, BCA, CAB)}{Total\ arm\ entries - 2}\right) 100 \ldots.(2)$$

Histological analysis

Following euthanasia with chloroform, rats were perfused with 10% formal saline, after which their brains were extracted. Coronal sections of the brain at the medial temporal lobe were then isolated for hippocampal morphology using routine haematoxylin and eosin staining (Szunyogova and Parson, 2016). Photomicrographs were captured using a Micro Video Capture^{R} camera mounted to an Olympus BH2 BHT microscope.

Statistical analysis

A one-way analysis of variance followed by the Tukey post hoc comparison test was used to evaluate the significance of differences between groups using GraphPad Prism 5 (San Diego, USA). Data are presented as the mean ± standard error of the mean (SEM). A p-value of ≤ 0.05 was considered statistically significant.

3. Results

Behavioural analysis

The cognitive functions of short-term non-spatial memory and short-term spatial memory were evaluated using the novel object recognition test and Y-maze test, respectively. Exposure to DV (group 2) resulted in significant decrease in DI compared to control ($p < 0.01$) (**Fig. 2.**). Conversely, co-exposure to DV and supplementation with vitamin C (VC) (groups 3-5) significantly enhanced DI relative to the DV-only group ($p < 0.01$). Note that VC supplementation had no discernible effect on DI compared to controls ($p < 0.1$), indicating that VC intervention improves short-term non-spatial memory. Values are mean ± SEM, * $p< 0.01$, Tukey post hoc, n = 5 per group.

DV-exposed rats in groups 2, 4 and 5 showed a significant decrease in the mean SA score compared to the control and group 3 rats (**Fig. 3.**). No significant difference is observed between the control and group 3. Groups 4 and 5 rats showed a significant difference in mean SA score compared to group 2. This indicates that vitamin C intervention is more effective at lower doses of DV exposure. Values are mean ± SEM, * $p< 0.01$, β $p < 0.001$, Tukey post hoc, n = 5 per group.

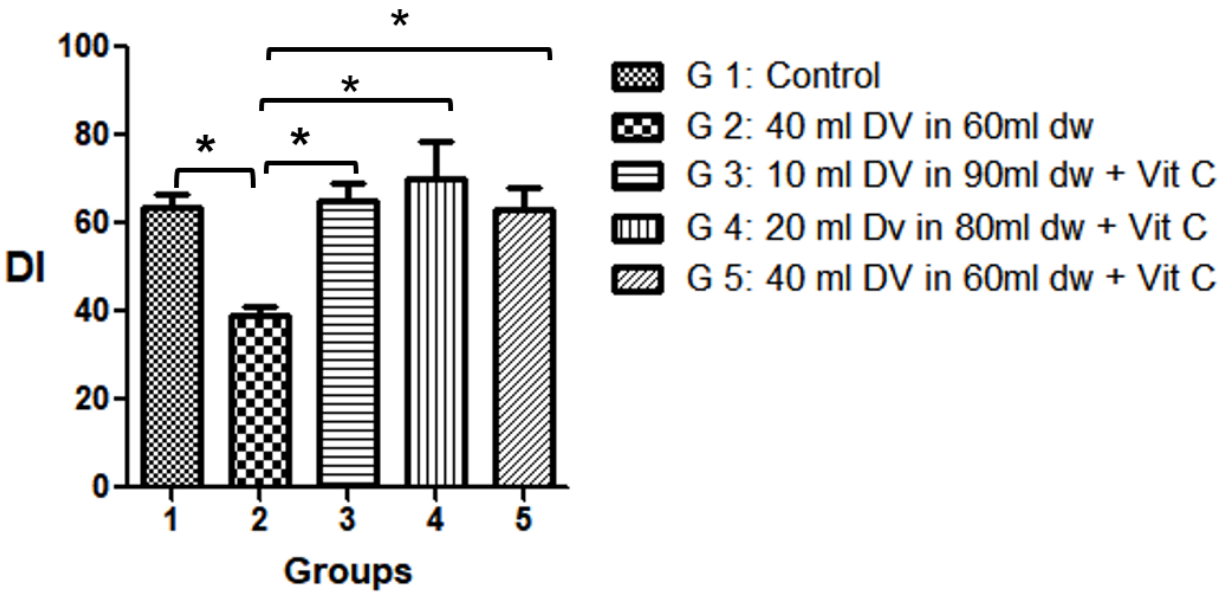

Fig. 2. Novel Object Recognition (NOR) Test: Mean Discrimination Index Scores.

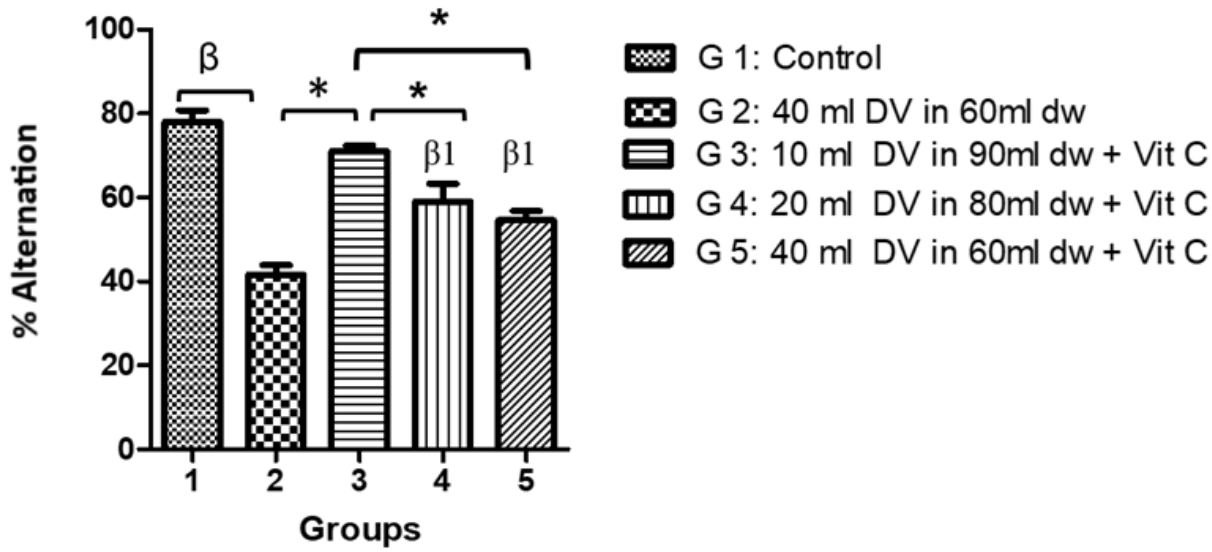

Fig. 3. Y-maze test: Mean Percentage Spontaneous Alternation

Histological analysis (Light-microscopic)

Results show the consequences of DV exposure in the dentate gyrus, CA3 and CA1 regions of the hippocampus (**Figures 4A, 5A, and 6A**).

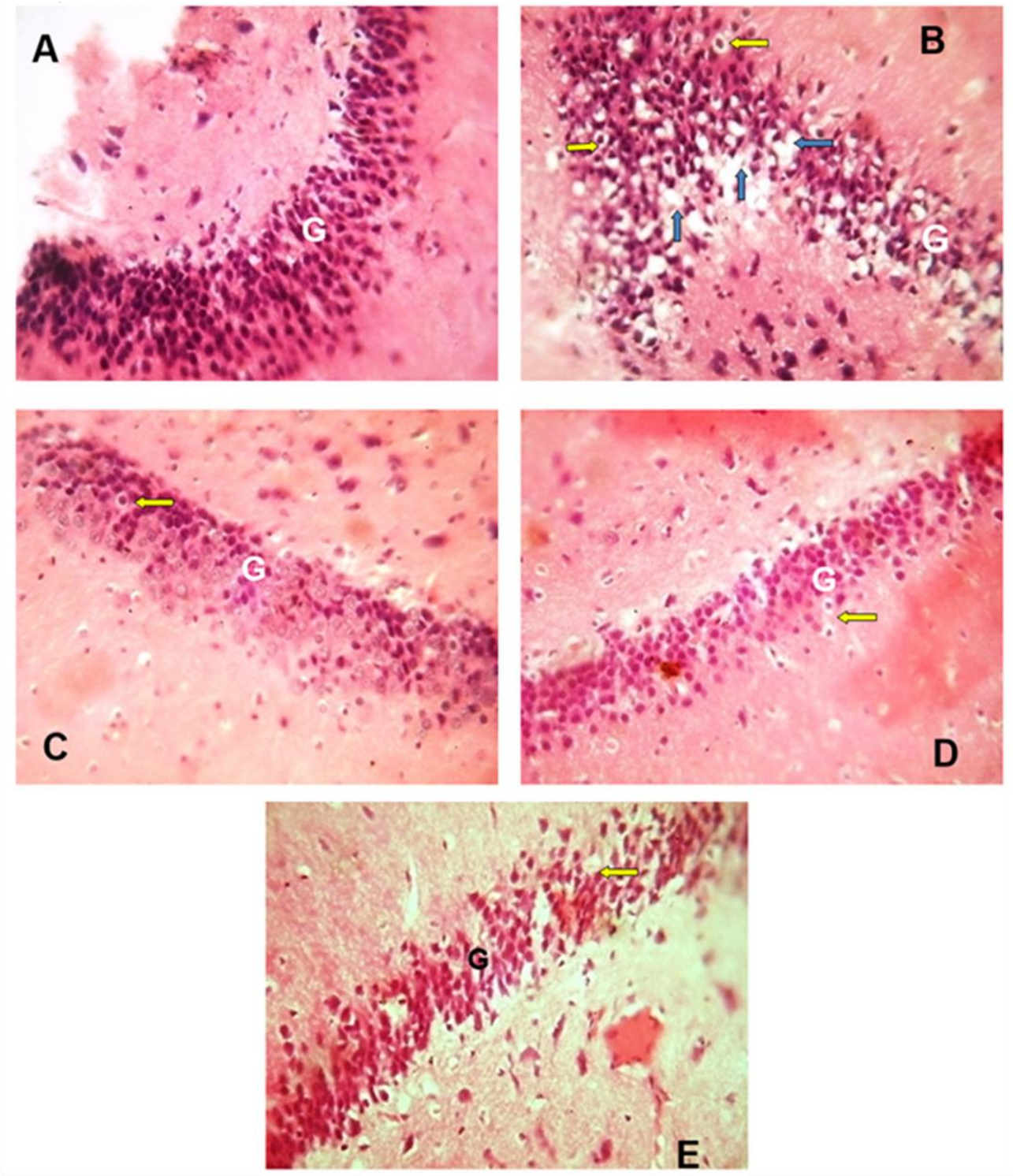

Fig.4. Dentate gyri of representative rats: Rats exposed to DV only (**B**), showed vacuolation (blue arrows) and numerous apoptotic degenerative neurons (yellow arrows). Note the relative reduction in the cell population in the granular layer compared to the control (**A**). Also, observe that the neurons in the G layers are relatively more in rats treated with vit-C (**C, D, and E**) compared to group 2 (**B**). Note the absence of vacuolation in rats that received vit-C. G, granular layer. H & E, mag. x 400.

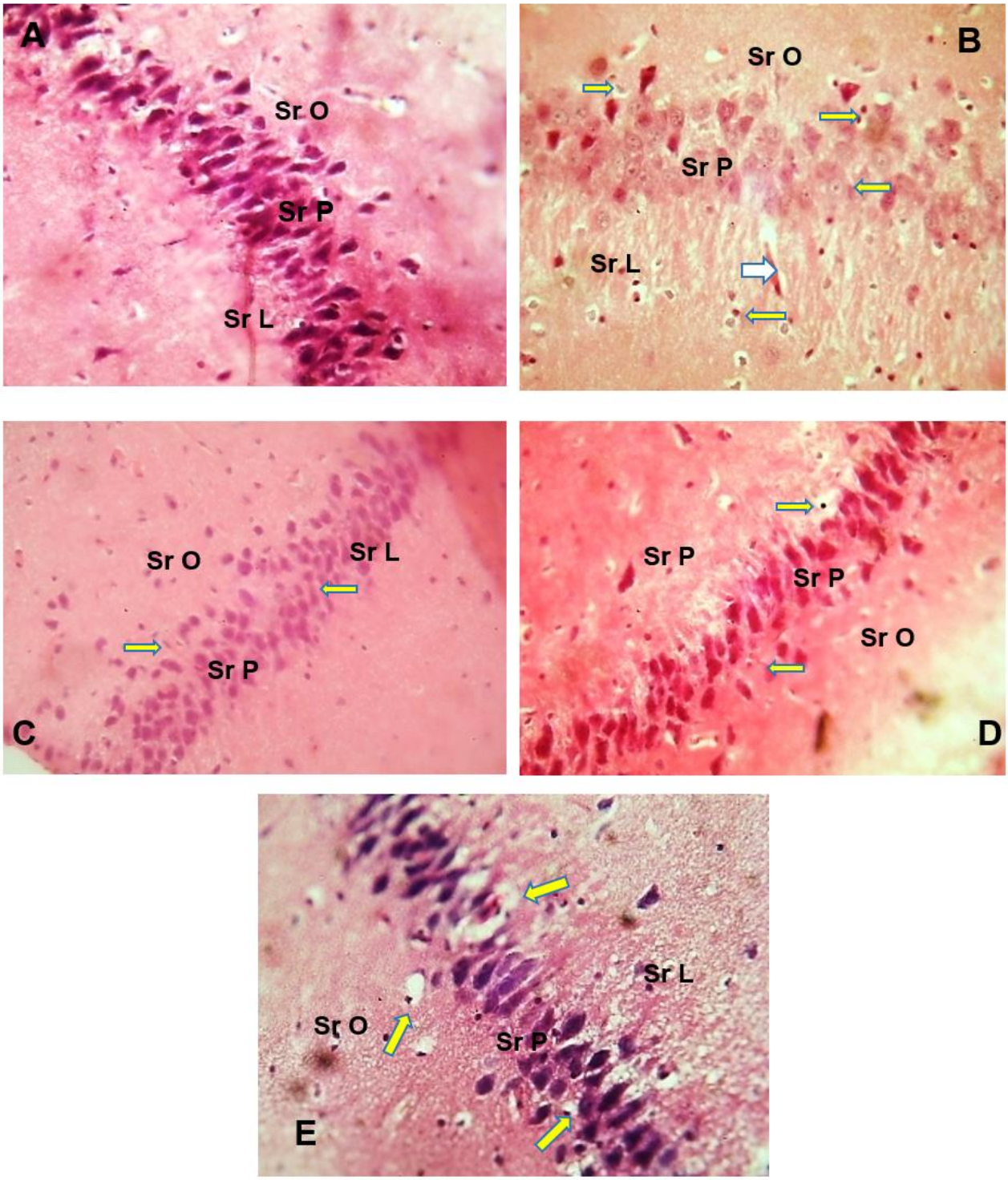

Fig. 5. CA3 regions of representative rats: DV exposure caused widely spread apoptotic (pyknotic) neurons (yellow arrows) and reduced neuronal density. Group 2 rats (**B**) also showed inflammation with loss of cyton and capillary dilation (white arrow). Rats that received vit-C (**C, D, and E**) showed no evidence of inflammation or loss of cyton. Widely spread pyramidal neurons were observed in group 2 because of cell death. Pyknotic degenerative changes were barely observed in GPs 3, 4 and 5 (**panels C, D and E respectively**) compared to GP 2 (**B**). StO, stratum oriens; StP, stratum pyramidales; StL, stratum lucidum. H & E, mag. x 400.

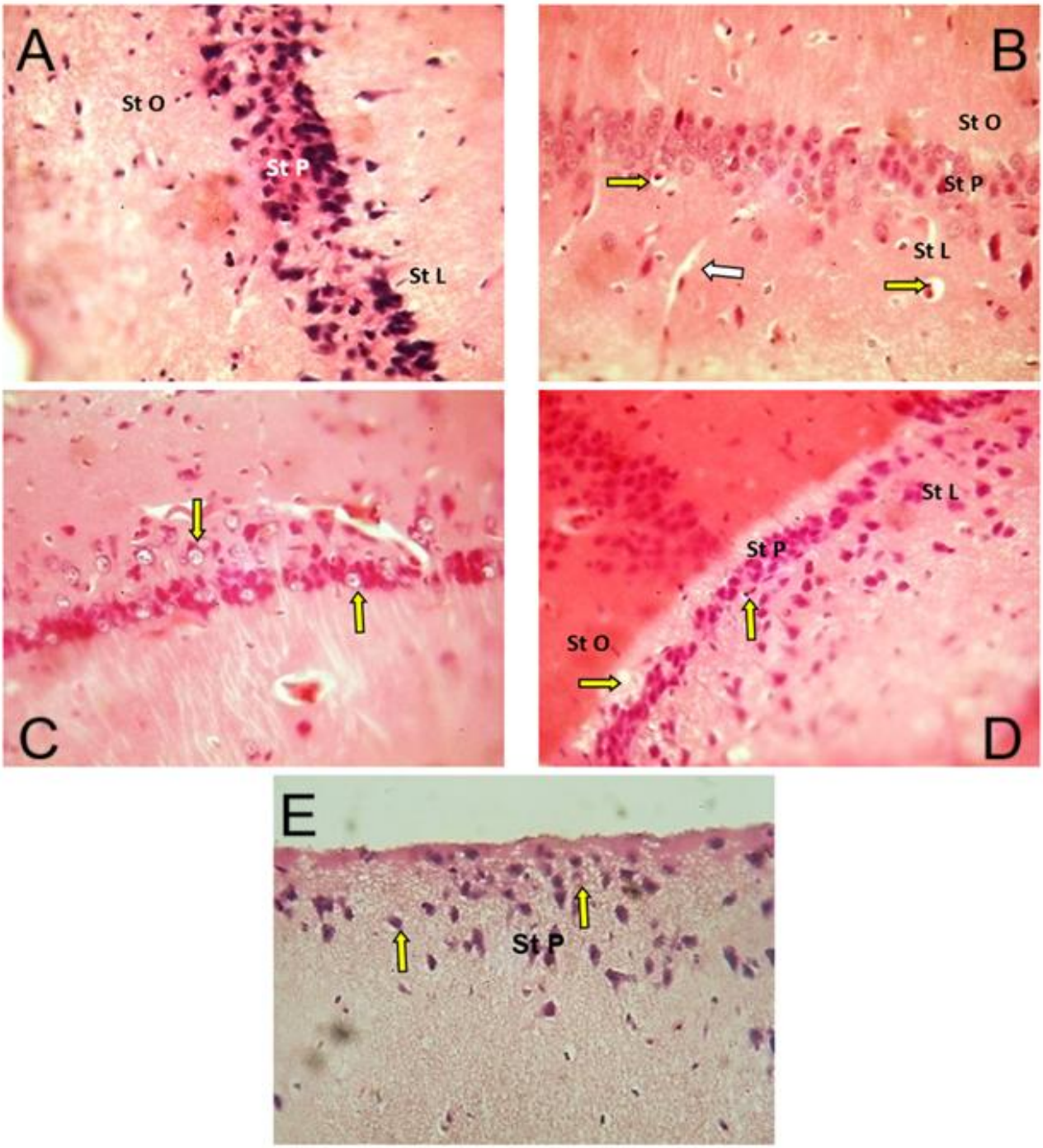

Fig. 6. CA1 regions of representative rats: Control (**A**) shows normal neuronal morphology in 3 strata. Rats exposed to 40 ml DV (**B**) showed loss of pyramidal cyton in the stratum pyramidales, widely spread pyknotic neurons (yellow arrows), dilated capillaries (white arrow), reduced neuronal density, and diffused vacuolation and inflammation. Rats that received vitamin C supplementation showed fewer pyknotic cells and degenerating neurons (**C, D, and E**). StO, stratum oriens; StP, stratum pyramidales; StL, stratum lucidum. H & E, mag. x 400.

4. Discussions

This study examined the neurotoxic effect of subacute DV inhalation on rat hippocampi and the potential of vitamin C to mitigate these effects. This work demonstrated the neurodegenerative consequences of dichlorvos on the hippocampus and the impact of ascorbic acid (vitamin C) on DV neurotoxicity (Figs. 2–6). The neurodegenerative changes observed were vacuolation, inflammation and apoptosis in the dentate neurons and pyramidal neurons (Figs 4B, 5B, and 6B); these findings are consistent with previous reports that align similar histopathological alterations in response to DV insults (Owoeye et al., 2014). This result also supported those of Abdel-Aziz et al. (2022), who demonstrated that vitamin supplementation lessened the extent of damage caused by dichlorvos-induced cytotoxicity in the rat hippocampus. Studies suggest that the pathophysiology of DV-elicited cytotoxicity is through phosphorylating the active centre of brain acetylcholinesterase, causing irreversible inhibition of the enzyme, resulting in acetylcholine building up in synapses and disrupting nerve function (Okoroiwu and Iwara, 2018; Mostafalou and Abdollahi, 2023). Dichlorvos, like other organophosphate insecticides, is known to trigger the production of reactive oxygen species (ROS) within the exposed organisms that result in oxidative damage, which can trigger cell, tissue, and organ damage (Farkhondeh et al., 2020). According to Sharma and Singh (2012), oxidative damage may be the cause of DV neurotoxicity, which results in necrotic cell death; however, we also observed apoptosis (Figs. 4, 5, and 6) characterised by nuclear condensation (Elmore et al., 2016), a fact that was missed out on in previous studies (Owoeye et al., 2014; Abdel-Aziz et al., 2022; Hart and David, 2022).

We found that giving ascorbic acid (vitamin C) to DV-exposed rats (DV + Vc) reduced hippocampal lesions compared to animals exposed to DV without supplements. The granule and pyramidal cells of the three regions showed mild to moderate neurodegeneration; this is consistent with the study of Owoeye et al. (2014), who reported partial amelioration of hippocampal lesions by vitamin C. Reports also suggest that ascorbic acid acts as a free radical scavenger (Gegotek and Skrzydlewska, 2022; Hart and David, 2022); thus, it is plausible that it reduced such oxidative reactions due to DV exposure (Sharma and Singh, 2012; Farkhondeh et al., 2020). Research has shown that hippocampal damage can have extensive effects on cognitive and emotional processes, as the hippocampus is a critical structure within the limbic system, playing a key role in learning, spatial cognition, emotion, social processing, and motivation (Somogyi, 2010; Todorov et al., 2019).

The results from this study on NORT and Y-Maze experiments show that animals in group 2 exhibited worse cognitive impairment after being exposed to dichlorvos only (**Figures 2 and 3**). These findings suggest that dichlorvos has a deleterious impact on learning and memory. Previous reports have indicated that memory decline results from neuronal cell loss along the intrinsic hippocampal pathway (dentate gyrus → CA3 → CA1 → subiculum) (Hainmueller and Bartos, 2020; Bartos, 2023). Neuronal loss may result in a decreased efficiency of neuronal receptivity, which could impair the encoding and consolidation of memories within the hippocampus. This could undermine the memory decline observed in rats treated with DV in that it causes neuronal cell loss in the dentate, CA3, and CA1 regions (4B, 5B, and 6B), thereby promoting memory deficit. Ascorbic acid directly affects the activity of neurotransmitters by modulating

their binding to their receptors and preventing neurodegeneration (Covarrubias-Pinto et al., 2015). Additionally, vitamin C promotes neuroplasticity, which improves the brain's capacity to reorganise and adapt in response to learning and memory formation and also in the development and strengthening of synapses, which facilitate signal transmission (Moretti and Rodrigues, 2022). Thus, in this study, supplementation with ascorbic acid following dichlorvos inhalation (DV + Vc groups) showed improved memory indices (figs 2 and 3). This study reveals that subacute DV inhalation adversely affects memory, which may be partially attenuated by ascorbic acid. However, although ascorbic acid showed some protective effects against dichlorvos-induced spatial and non-spatial memory impairments, the incomplete recovery, notably at higher doses, implies persistent damage to memory formation mechanisms.

This study provides conclusive evidence that vitamin C supplementation can effectively mitigate the detrimental effects of dichlorvos on the hippocampus of Wistar rats. Consistent with previous findings (Akamo et al., 2025), dichlorvos exposure led to pronounced neuronal degeneration within the hippocampus, characterized by a significant increase in apoptotic neurons and a corresponding decrease in neuronal density. This was reflected in the poor performance observed in both the Y-maze and novel object recognition tests (figures 2 and 3), which indicate cognitive deficits (Mostafalou and Abdollahi, 2023). However, co-administration with vitamin C significantly attenuated these obtuse effects. Vitamin C treatment was associated with improved performance in both behavioural tasks, suggesting a significant restoration of cognitive function in the treated rats. This could be plausible due to vitamin C's ability to reduce ROS assault since ROS-induced damage is primarily the mechanism of OP-triggered cellular injury. There was considerably less neuronal damage in the vitamin C-treated rats compared to DV-exposed rats without vitamin C supplementation, suggesting that vitamin C reduces the neurotoxic effects of DV, potentially protecting neuronal integrity and lowering the risk of neurodegeneration.

Conclusions

Despite the valuable insights obtained from this study, several limitations require consideration. While our T-maze and NOR tests could only assess certain critical aspects of cognition, specifically spatial working memory and recognition memory, other aspects of cognition, such as spatial reference memory, executive functions, attention, and cognitive flexibility, remain unexplored in this study. Future studies could benefit from incorporating more comprehensive cognitive tests, such as the Morris water maze, Barnes maze, and the radial arm maze, to provide a more nuanced understanding of how undue dichlorvos exposure impacts cognitive function. It would be desirable if these assessments were automated. Software-automated tests reduce animal handling and stress while increasing researcher efficiency and productivity by expediting data collection, analysis, and interpretation. Nevertheless, our findings suggest that vitamin C may play a neuroprotective role against hippocampal degeneration and cognitive impairment induced by organophosphate-based insecticides in rats.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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