Short-Term Sensorimotor and Cognitive Changes Induced by Acute Chlorpyrifos Exposure in Wistar Rats: Ameliorative Effect of Vitamin E

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Abstract: Background: Acute chlorpyrifos exposure has been shown to induce long-term neurobehavioural and cognitive changes in animal models and humans. Although, inhibition of acetylcholinesterase activity is the main mechanism of CPF toxicity, the induction of oxidative stress has been shown to play an important role in exacerbating the toxicity. The present study was therefore aimed at evaluating the mitigating effect of antioxidant vitamin E on short-term changes in sensorimotor and cognitive behaviours induced by acute CPF exposure in Wistar rats. **Methods:** Forty adult male Wistar rats were divided into 4 groups of 10 rats in each group. Group I was administered C/oil (2 mL kg^{-1}) while group II was dosed with vitamin E only (75 mg kg^{-1}). Group III was exposed to CPF only (42.5 mg kg^{-1}~50% of LD$_{50}$). Group IV was administered vitamin E (75 mg kg^{-1}) and then exposed to CPF (42.5 mg kg^{-1}), 30 min later. The regimens were administered once by gavage. Neurobehavioural parameters such as motor coordination, efficiency of locomotion, neuromuscular coordination, learning and short-term memory were evaluated at various times during the study using the appropriate devices. The animals were sacrificed at the end of the 11th week after the administration of the regimen and the brain was evaluated for Malonaldehde (MDA) concentration and AChE activities. **Results:** There were short-term impairments in motor coordination, efficiency of locomotion, neuromuscular coordination, learning and memory and decrease in the level of brain MDA and AChE in the group exposed to CPF only which were ameliorated by Vitamin E pretreatment. **Conclusion:** The study has shown that vitamin E mitigated the short-term sensorimotor and cognitive changes induced by acute chlorpyrifos exposure in Wistar rats, partly due to its antioxidant and acetylcholinesterase restoration properties.

Key words: Acute chlorpyrifes, cognitive change, oxidative stress, malonaldehde

INTRODUCTION

There is growing evidence from clinical, epidemiological and experimental studies that exposure to pesticides may induce some neurological (Kamel and Hoppin, 2004), neuropsychiatric (London *et al.*, 2005) neurobehavioural (Colosio *et al.*, 2003) and neurodegenerative (Priyadarshi *et al.*, 2001; Brown *et al.*, 2006) changes. Organophosphate (OP) insecticides which accounts for most worldwide usage in agriculture and public health (Casida and Quistad, 2004) are known to cause long-term neurotoxic effect due to its ability to inhibit Aacetylcholinetsearse (AchE) activity (Baldi *et al.*, 2010). The occurrence of chronic neurobehavioral impairment from acute OP exposure has been well documented (Savage *et al.*, 1988; Rosenstock *et al.*, 1991; Pope *et al.*, 1992; Steenland *et al.*, 1994; Wesseling *et al.*, 2002; Stallones and Beseler, 2002; Canadas *et al.*, 2005; Ambali *et al.*, 2010).

Chlorpyrifos, a broad spectrum OP insecticide, remains one of the most extensively used in agriculture, industry and households (Slotkin, 2004) despite the restriction placed on some of its domestic applications by the United States Environmental Protection Agency in 2000. Subcutaneous administration of CPF has been shown to produce extensive long-lasting AChE inhibition, because of its slow rate of delivery (Richardson, 1995). Biochemical studies have also shown that large acute single dose CPF exposure produced persistent inhibition of AchE activities that lasts several weeks (Bushnell *et al.*, 1993; Carvajal *et al.*, 2007; Lopez-Crespo *et al.*, 2007) apparently due to its high lipid solubility and tissue persistence. Although, inhibition of AChE remains the main mechanism of CPF-induced acute neurotoxicity, other non-cholinergic mechanism including the induction of oxidative stress is been increasingly implicated (Vidyasagar *et al.*, 2004; Gultekin *et al.*, 2007; Ambali *et al.*, 2010). Oxidative stress which results

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from imbalance of oxidants and antioxidants in favour of the former causes cellular damage (Sies, 1991). Although the body system is equipped with antioxidant machineries to combat the danger posed by Reactive Oxygen Species (ROS) under normal circumstance. However, during oxidative stress as previously reported in CPF poisoning, the body's antioxidant systems are overwhelmed by the oxidants. Under this circumstance, provision of exogenous antioxidants becomes imperative to assist the body neutralizes the damaging effect of ROS. Vitamin E is a lipid soluble chain-breaking antioxidant vitamin that has been demonstrated to mitigate the damaging effect of ROS in the cell membrane. We have earlier demonstrated that short-term neurobehavioural and cognitive changes induced by acute CPF exposure in Wistar rats are ameliorated by Vitamin C (Ambali *et al.*, 2010). Therefore, the objective of the present study was to evaluate the ameliorative effect of vitamin E on short-term neurobehavioural and cognitive changes induced by acute CPF exposure in Wistar rats.

MATERIALS AND METHODS

Experimental animals: Twenty eight adult male Wistar rats were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria and housed in metallic cages in the Toxicology Laboratory of the Department. The rats were fed standard on rat chow and water was provided ad libitum. The animals were allowed to acclimatize in the environment for at least two weeks before the commencement of the study.

Chemicals: Chlorpyrifos (TERMICOT®, Saberos Organics Gujarat Ltd, India), a 20% emulsifiable concentrate was reconstituted using corn oil to 10% stock solution. Commercial grade Vitamin E (100 mg/caplet, Pharco Pharmaceutical Industries Ltd., Alexandra, Egypt) was reconstituted in corn oil (50 mg mL^{-1}).

Animal treatments schedule: The 28 male Wistar rats were divided into four groups of 7 animals in each group. Group I (C/oil) was administered corn oil (2 mL kg^{-1} b.w.) while group II was administered Vitamin E (75 mg kg^{-1} b.w). Group III (C/oil) was administered CPF [42.5 mg kg^{-1} b.w., i.e. ~50% of LD$_{50}$ of 85 mg kg^{-1} as determined by Ambali (2009)]. Group IV (VE+CPF) was administered Vitamin E (75 mg kg^{-1} b.w.), followed by CPF (42.5 mg kg^{-1} b.w.), 30 min later. These regimens were administered once by gavage. The animals were monitored for clinical signs, death and periodic neurobehavioural changes. At the end of week 11 after

exposure to the regimens, the rats were humanely sacrificed using jugular venesection after initial light chloroform anaesthesia and the freshly obtained brain samples were evaluated for malonaldehyde concentration and acetylcholinesterase activity.

Evaluation of the effect of treatments on motor coordination: The assessment of motor coordination was performed using the beam walk performance task as described by Ambali *et al.* (2010). The assessment was done on day 0, weeks 3, 7 and 11.

Evaluation of the effect of treatments on neuromuscular coordination: The effect of the treatments on neuromuscular coordination was assessed using the incline plane apparatus as described by Ambali *et al.* (2010). This procedure was carried out on each animal from all the groups on day 0, weeks 3, 7 and 11 of the study.

Evaluation of the effect of treatment on efficiency of locomotion: For the assessment on ladder walk, every rat from each group was encouraged to walk across a black wooden ladder (106×17 cm) with 0.8 cm diameter rungs and 2.5-cm spaces between them. The number of times each rat missed a rung was counted by one rater on each side (Petrich, 2006). Performance on ladder walk was evaluated on Day 0, weeks 3, 7 and 11. Two trials were performed for each testing section.

Evaluation of the effect of treatments on learning and short-term memory acquisition: The effect of the treatments on learning acquisition and short-term memory in rats was assessed at 48 and 24 h, respectively, to the termination of the experiment using the step-down inhibitory avoidance device as described by Zhu *et al.* (2001).

Brain tissue preparation: The whole brain tissue was carefully dissected and a known weight of the brain sample from each animal in the four groups was homogenized in a known volume of ice cold phosphate buffer to obtain a 10% homogenate. This was then centrifuged at 3,000×g for 10 min to obtain the supernatant. The supernatant was then used to assess the levels of protein, MDA, AChE in the brain sample.

Evaluation of brain malonaldehyde concentration: For the determination of brain lipoperoxidative changes, the double heating method of Draper and Hadley (1990) as modified by Freitas *et al.* (2005) was used. The concentration of MDA in the brain tissues was calculated

by the absorbance coefficient of MDA-TBA complex $(1.56 \times 105 \text{ cm}^{-1} \text{ M}^{-1})$ and expressed as nmol mg^{-1} of tissue protein. Tissue protein was analyzed using the Lowry method (Lowry *et al.*, 1951).

Evaluation of brain acetylcholinesterase activity: The brain acetycholinesterase activity was determined according to the method of Ellman *et al.* (1961). This method employs Acetylthiocholine (ATChI) as a synthetic substrate for AChE. ATChI is broken down to thiocholine and acetate by AChE and thiocholine is reacted with Dithiobisnitrobenzoate (DTNB) to produce a yellow color. The quantity of yellow color which develops over 30 min measures the activity of AChE using a UV spectrophotometer (T80$^+$ UV/VIS Spectrometer, PG Instruments Ltd., UK) at 405 nm. The AChE activity was expressed as nmol/min/mg tissue protein.

Statistical analysis: Data were obtained as mean ±SEM. The data obtained from beam walk, ladder walk and incline plane tests were analyzed using the repeated measure analysis of variance followed by Tukey's test. The data from step-down avoidance test and those obtained from the estimation of the levels of malonaldehyde and acetylcholinesterase were analyzed using one-way analysis of variance followed by Tukey's posthoc test (Manjunath and Telles, 2004). Values of p<0.05 were considered significant.

RESULTS

Clinical signs: No signs of toxicity were observed in the C/oil and VE groups. Toxic signs recorded in the CPF group include somnolence, ataxia, dyspnoea, ruffled fur, lacrimation, tremor, diarrhea, muscular weakness and death in three animals. The clinical signs were observed at week 1, while death occurred at weeks 11 and 12 post-exposure. Rats in the VE+CPF group manifested lacrimation, mild tremor and ruffled fur.

Effect of treatments on motor coordination: The dynamics of beam walk length showed a progressive increase in the width at which rats in the CPF group slipped off the beam up to week 7 follow by a slight declined at week 11. Generally, the width at which CPF group slipped off the beam increased significantly (p<0.01) at week 7 when compared to those recorded at day 0, weeks 3 and 11. There was a significant (p<0.05) increase in the width at which VE+CPF group slipped off the beam at week 11 compared to day 0. There was no significant difference (p>0.05) in the width of slip in either the C/oil or VE group in between the weeks.
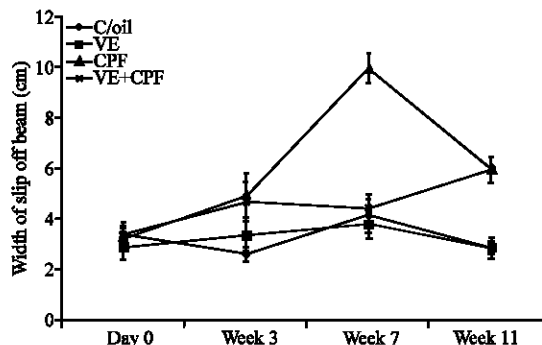

Fig. 1: Effect of acute administration of corn oil (C/oil), chlorpyrifos (CPF) and/or Vitamin E (VE) on short-term changes in the dynamics on motor coordination in wistar rats

At week 3, there was a significant (p<0.05) increase in the width at which the CPF group slipped off the beam when compared to C/oil and VE groups, respectively. There was a significant (p<0.01) increase in the width at which the CPF group slipped off the beam at weeks 7 and 11 when compared any of the other groups (Fig. 1).

Effect of treatments on efficiency of locomotion: The number of times the CPF group missed the ladder rungs was significantly higher (p<0.05) at day 0 when respectively compared to weeks 3, 7 and 11. There was no significant change (p>0.05) in the number of missed rungs in the C/oil, VE and VE+CPF groups in between the weeks of evaluation.

At day 0, there was no significant change (p>0.05) in the number of times the animals in all the groups missed the ladder rungs in between the groups. At week 3, there was a significant (p<0.05) decrease in the number of times the CPF group missed the ladder rungs when respectively compared to C/oil, VE and VE+CPF groups. At week 7, the number of times the CPF group missed the rungs decreased significantly (p<0.01) compared to C/oil or VE group but no significant change (p>0.05) compared to VE+CPF group. At week 11, only the VE group showed a significant (p<0.05) increase in the number of times it missed the rungs compared to the CPF group (Fig. 2).

Effect of treatments on neuromuscular coordination: There were no significant changes (p>0.05) in the angles of slip in between the group throughout the evaluation period except at week 11 when the angle at which rats in the VE+CPF group slipped off the incline plane was significantly higher (p<0.05) compared to CPF group.
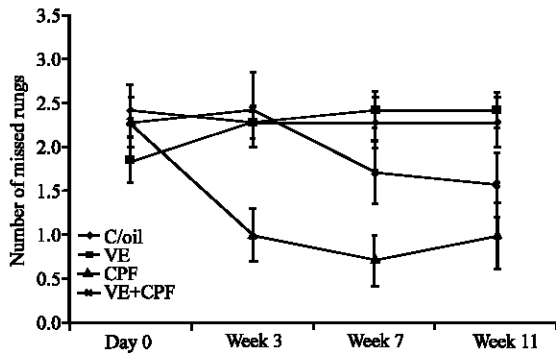
Fig. 2: Effect of acute administration of corn oil (C/oil), chlorpyrifos (CPF) and/or Vitamin E (VE) on efficiency of locomotion in ladder walk dynamics in wistar rats
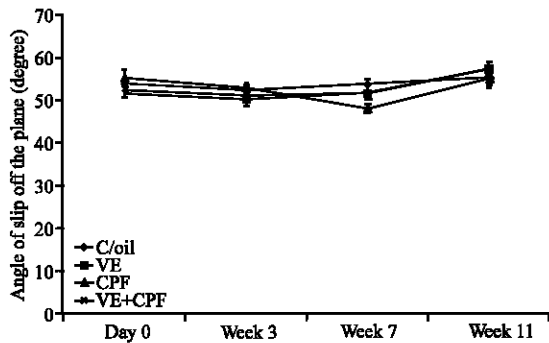

Fig. 3: Effect of acute administration of corn oil (C/oil), chlorpyrifos (CPF) and/or Vitamin E (VE) on neuromusclular coorelination in ladder walk dynamics in wistar rats
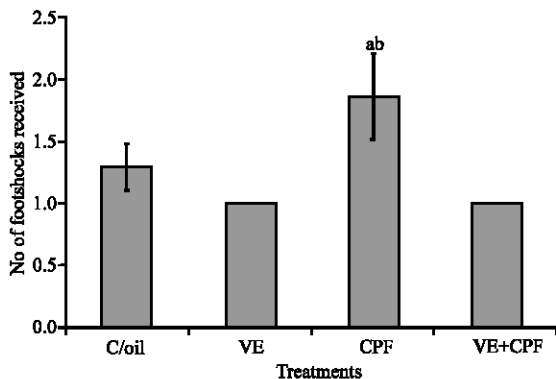

Fig. 4: Effect of acute administration of corn oil (C/oil), chlorpyrifos (CPF) and/or Vitamin E (VE) on short-term learning acquisition in wistar rats. [ab]$p<0.05$ versus VE and VE+CPF groups, respectively
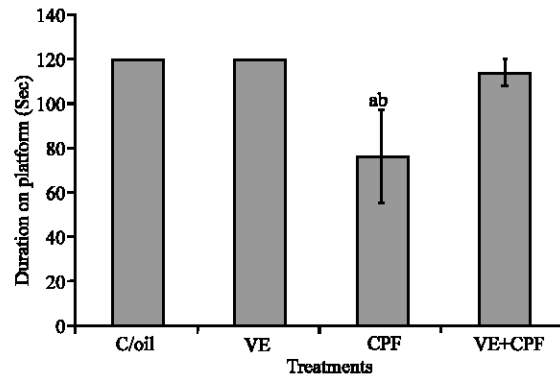

Fig. 5: Effect of acute administration of corn oil (C/oil), chlorpyrifos (CPF) and/or Vitamin E (VE) on short-term memory in wistar rats. [ab]$p<0.05$ versus S/oil and VE groups, respectively

The dynamics within the group showed a significant increase in the angle at which the VE group slipped off the beam at day 0 compared to week 11 ($p<0.05$), week 3 versus week 7 ($p<0.05$) and week 3 versus week 11 ($p<0.01$). The CPF group showed a significant decrease ($p<0.05$) in the angle of slip at week 7 compared to either day 0 or week 11 (Fig. 3).

Effect of treatments on learning acquisition: There was a significant ($p<0.05$) increase in the number of footshocks received by rats in the CPF group compared to the VE or VE+CPF group. Although not significant, the mean number of foot-shocks received by rats in the CPF group increased by 32%, when compared that of C/oil group (Fig. 4).

Effect of treatments on short-term memory: There was a significant ($p<0.05$) decrease in the duration of stay on the platform in the platform in the CPF group compared to either S/oil or VE group. Although not significant ($p>0.05$), the duration of stay on the platform in the CPF group was 33% lower relative to the VE+CPF group (Fig. 5).

Effect of treatments on brain malonaldehyde concentration: There was a significant increase ($p<0.05$) in the brain MDA concentrations in the CPF group when compared to the C/oil or VE group. Although not significant ($p>0.05$), the mean MDA concentration in CPF group was 19% higher than VE+CPF group (Fig. 6).

Effect of treatments on acetylcholinesterase activity: There was a significant decrease ($p<0.05$) in the brain AchE activity in the CPF group compared to the VE
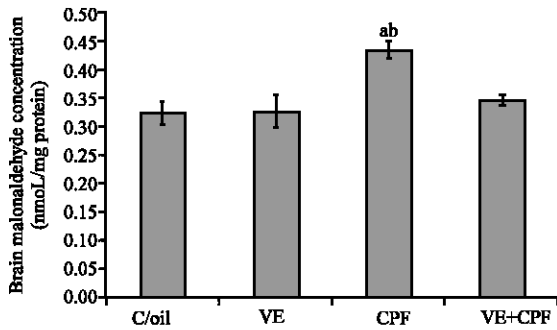
Fig. 6: Effect of acute administration of corn oil (C/oil), chlorpyrifos (CPF) and/or Vitamin E (VE) on brain malonaldehyde concentration in wistar rats. [ab]$p<0.05$ versus C/oil and VE groups, respectively
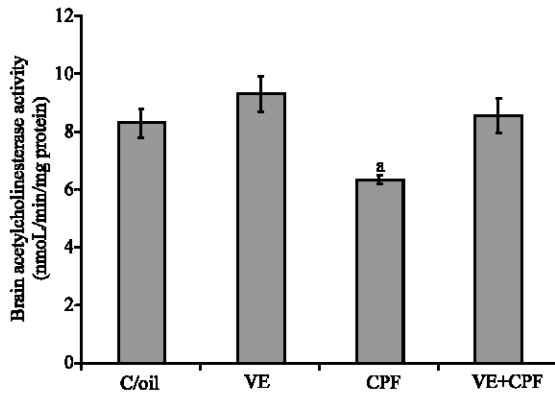

Fig. 7: Effect of acute administration of corn oil (C/oil), chlorpyrifos (CPF) and/or Vitamin E (VE) on brain acetylcholinesterase concentration in wistar rats. [a]$p<0.05$ versus VE group

group. Although not significant, the brain AChE activity in the CPF group relatively decreased by 23 and 26% when compared to C/oil and VE+CPF groups, respectively (Fig. 7).

DISCUSSION

The clinical signs observed in the CPF group may be due to the inhibition of enzyme AChE activity, leading to accumulation of ACh in the cholinergic receptors in the peripheral and central nervous system. The accumulation of ACh in the cholinergic receptors leads to muscarinic, nicotinic and central nervous cholinergic syndromes (Eaton *et al.*, 2008). The reduction of severity of clinical signs in group pretreated with Vitamin E may be due to increase AChE activity as recorded in the present study. In addition, the ability of Vitamin E to increase

paraoxonase activity (Jarvik *et al.*, 2002), resulting in rate of detoxification of the OP insecticide may have contributed to the reduction in the severity of toxic manifestations in the VE+CPF group.

The present study showed a significant deficit in the motor coordination of rats in the CPF group when the initial performance was compared to that recorded at termination. This finding is similar to those recorded by Ambali *et al.* (2010). This may be due to damage to the portion of the brain responsible for motor coordination, probably due to oxidative stress as exemplified by increased MDA concentration in the group. Pretreatment with Vitamin E was shown to have significantly improved the motor coordination impaired by CPF. This reaffirms the fact that oxidative stress is partly involved in the short-term motor coordination deficit induced by acute CPF exposure.

The deficit in the efficiency of locomotion in the CPF group was manifested by the decrease in the number of missed rungs during the ladder walk. This is similar to what we observed in a previous study following chronic CPF exposure in rats (Ambali, 2009). Similarly, slowness of movement is one of the extrapyramidal symptoms observed following exposure to non specific agricultural pesticides (Ritz and Yu, 2000; Alavanja *et al.*, 2004). The locomotor deficits recorded in the CPF group may be partly due to either oxidative damage to the muscle or impairment of neuronal transmission as a result of paralysis of the neuromuscular junction apparently resulting from prolonged AChE inhibition. Vitamin E was able to improve the efficiency of locomotion partly due to its antioxidant and AChE restoration properties which ultimately improve neuronal activity.

The performance on the incline plane is used to access neuromuscular coordination. The angles at which rats in CPF group slipped off the incline plane were comparatively lower than the other groups, indicating an apparent deficit, hence impaired neuromuscular coordination. This agreed with our previous result (Ambali *et al.*, 2010). The impairment of neuromuscular coordination may be due to increase in brain lipoperoxidative changes induced by CPF which alters the morphological and functional capacity of the brain region involved in neuromuscular coordination. Verma and Srivastava (2001) and Ambali *et al.* (2010) reported increased oxidative damage to the brain following CPF exposure. Similarly, the reduced AChE activity may have partly played a role in the impairment of neuromuscular coordination recorded in the CPF group, since alterations in ACh metabolism may also alter neuronal activity. The improvement in incline plane performance in the group pretreated with Vitamin E indicates improved

neuromuscular coordination. This may be due to amelioration of CPF-induced lipoperoxidative damage to the brain resulting in protection of neuronal membrane integrity, hence their activities. Similarly, the restoration of brain AChE activity by the Vitamin E may have partly contributed to the improved neuromuscular coordination in this group.

The increase in the number of footshocks received by rats exposed to CPF only compared to other groups in the step down avoidance instrument revealed a deficit in learning acquisition in this group. The result obtained from the present study agreed with earlier findings following.

CPF exposure (Canadas *et al.*, 2005; Gultekin *et al.*, 2007; Prendergast *et al.*, 2007; Ambali, 2009; Ambali *et al.*, 2010). This deficit may have arisen following neuronal damage partly due to induction of oxidative stress in the relevant portions of the brain responsible for learning acquisition, especially in the cerebral cortex.

Memory impairment in the group exposed to CPF only in the present study had been reported in previous studies (Canadas *et al.*, 2005; Ambali *et al.*, 2010). Similarly, epidemiological studies have shown that both acute and chronic exposure to CPF induced long-term neurocognitive deficit (Yokoyama *et al.*, 1998).

The cognitive decline may have been partly due to oxidative damage to the portions of the brain responsible for memory, especially, the hippocampus. Similarly, inhibition of AChE activity may have played a role in the cognitive decline. Several studies have linked cognitive decline to alteration in ACh metabolism (Overstreet, 1984; Sachdev *et al.*, 1998). The alteration in AChE activity may also be linked to induction of oxidative stress, since hydroxyl radical has been shown to cause significant reduction in AchE activity in the rat brain (Tsakiris *et al.*, 2000). The improvement in the cognitive performance following pretreatment with Vitamin E underscores the significance of oxidative stress in the pathogenesis of CPF-induced cognitive decline. The neuroprotective effect of Vitamin E may have been due to its antioxidant property of the vitamin. Furthermore, the ability of the vitamin to improve the AChE activity may have aided in the improvement of cognition impaired by CPF.

The significant increase in brain lipid peroxidation manifested as increased MDA concentration in CPF group suggests that oxidative stress may have been partly involved in the molecular mechanism of neurobehavioral deficits observed in the present study which is in agreement with findings from previous studies (Gultekin *et al.*, 2007; Ambali *et al.*, 2010).

Vitamin E has been shown by the present study to apparently reduce the MDA concentration due to its antioxidant effects which is in agreement with previous studies (Ahmed *et al.*, 2010).

The present study has also shown that acute CPF exposure also resulted in relatively short-term AChE inhibition. This finding agrees with Canadas *et al.* (2005) and Ambali *et al.* (2010). The AChE inhibition which persisted long after the exposure, may be due to high lipid solubility and persistence of CPF in the brain tissue. The AChE inhibition may have played some role in the neurobehavioral and cognitive changes recorded in the CPF group. Vitamin E was shown by the present study to reactivate the AChE activity depressed by CPF. Yavuz *et al.* (2004) demonstrated AChE restoration properties of Vitamin E following OP exposure. Similarly, Ambali *et al.* (2010) have also shown that vitamin C also improves AChE activity following inhibition by OP compound.

In conclusion, the present study has shown that Vitamin E mitigates short-term sensorimotor and cognitive changes induced by acute CPF exposure in Wistar rats, partly due to mitigation of brain lipoperoxidative changes and restoration of AChE activity.

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