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Full Length Research Paper

Chronic chlorpyrifos-induced sensorimotor and cognitive deficits in Wistar rats- Reparation by Vitamin C

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Abstract

The objective of the present study was to evaluate the role of oxidative stress and mitigating effect of vitamin C on sensorimotor and cognitive changes induced by chronic chlorpyrifos (CPF) exposure in male Wistar rats. Twenty adult male Wistar rats divided into 4 groups of 5 animals each served as subject for this study. Group I (S/oil) was administered with soya oil (2 ml/kg) while group II (VC) was given vitamin C (100 mg/kg); group III was dosed with CPF (10.6 mg/kg~1/8th of the LD_{50}); group IV was pretreated with vitamin C (100 mg/kg) and then exposed to CPF (10.6 mg/kg), 30 min later. The regimens were administered orally by gavage once daily for a period of 17 weeks. The animals were evaluated for toxic signs and neurobehavioral parameters. At the end of the period, the whole brain samples were evaluated for the levels of malonaldehyde (MDA) and activities of superoxide dismutase (SOD), catalase (CAT), and acetylcholinesterase (AChE). The result showed that toxic signs and the impairments of coordinated gaits, neuromuscular coordination, learning and short-term memory induced by CPF exposure were mitigated by vitamin C. The increased MDA concentration in the CPF group was significantly reduced by vitamin C pretreatment. The activities of SOD, CAT and AChE which were reduced by CPF exposure significantly improved in vitamin C pretreated group. In conclusion, this study has shown that vitamin C mitigates sensorimotor and cognitive deficits induced by chronic CPF exposure in Wistar rats apparently due to its AChE restoration and antioxidant properties.

Keywords: Chlorpyrifos, Neurobehavioral deficit, Oxidative stress, Acetylcholinesterase, Antioxidant, Reparation, Vitamin C

INTRODUCTION

There has been a global increase in pesticides usage due to the compelling need to feed the ever-increasing human and animal populations, and to reduce the incidence of food and vector-borne diseases (Gubler, 1998). These health and economic benefits of pesticide usage are achieved not without simultaneous potential health risks and adverse health outcomes in non-target species, including man. Association between acute exposure to pesticides and neurotoxicity is well known (Lotti, 2000). On the contrary, the potential effects of chronic low-level exposures are less well established (Alavanja et al., 2004; Ambali et al., 2010c, 2012). Oxidative stress has been implicated in pesticides-related neurotoxicity (Abdollahi et al., 2004).

Organophosphate (OP) compounds are one of the most widely used insecticides accounting for about 50%

List of abbreviations: AChE, acetylcholinesterase; CAT, catalase; CPF, chlorpyrifos; MDA, malonaldehyde; OP, organophosphate; PBS, phosphate buffer saline; ROS, reactive oxygen species; S/oil, soya oil; VC, vitamin C.

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Of the global insecticide use (Casida and Quistad, 2004). It should be noted, however, that while a large number of human and animal studies have focused on the long-term consequences of acute OP exposure, relatively little attention has been paid to the subject of chronic, "low-level" OP exposures that are not associated with apparent systemic toxicity (Ray and Richards, 2001; Rothlein et al., 2006). This may be of particular concern given the widespread use of OP insecticides (and consequent human and animal exposure) in household, agricultural, and commercial environments worldwide (Terry et al., 2007). The issue of repeated, subthreshold exposures to Ops is very important, since detectable levels can remain in the environment (particularly indoor environments) for extended periods after application (Krieger et al., 2001), thereby posing risk for low-level exposure (Terry et al., 2003). Studies in humans showed neurological, cognitive and psychomotor impairments following cumulative exposure to Ops and organochlorines in people from agricultural communities, without history of acute poisoning (Kamel and Hoppin, 2004; Kamel et al., 2007). Neurobehavioural changes following low-dose OP exposure have been reported in sheep farmers (Stephens et al., 1995), greenhouse workers (Bazylewicz-Walczak et al., 1999), tree-fruit workers (Fiedler et al., 1997), and farm workers (Kamel et al., 2003). These studies have found deficits in measures of sustained attention, information processing, motor speed and coordination.

Chlorpyrifos (CPF) is a chlorinated OP insecticide that has enjoyed widespread use in agricultural and domestic pest control (Steenland et al., 2000; Ambali et al., 2009). It is of public health importance as CPF residues have been detected in poultry egg, meat, cow milk and milk products (Rawat et al., 2003). Exposure to CPF at doses that did not result in overt clinical symptoms has been reported among pesticide applicators and other farm workers (Farahat et al., 2010) thereby constituting an important source of occupational hazards to these groups of individuals. The mechanism of acute neurotoxicity of CPF, like many other OP insecticides, is related to inhibition of acetylcholinesterase (AChE), an essential enzyme that terminates acetylcholine activity in the nervous system. However, neurobehavioural and cognitive deficits have been observed following repeated low-dose CPF exposure (Stamper et al., 1988; Sanchez-Santed et al., 2004) that cannot be attributed to the usual AChE inhibition and muscarinic receptor binding (Pope et al., 1992; Chakraborti et al., 1993). Therefore, other mechanisms including the induction of oxidative stress have been linked with repeated low dose CPF-induced neurobehavioural deficit (Abou-Donia, 2003; Gultekin et al., 2007; Prendergast et al., 2007).

Oxidative stress such as those induced by CPF results in undesirable oxidation, causing membrane damage, protein modification, DNA damage and cell death induced by DNA modification and lipid peroxidation (Singh et al., 2004). However, the body is endowed with enzymatic and non-enzymatic antioxidant systems to counter the lipoperoxidative changes induced by reactive oxygen and nitrogen species. In case of increased and accelerated oxidative challenge associated with CPF exposure (Gultekin et al., 2001; Ambali et al., 2010 a-c, 2011), these antioxidant systems are overwhelmed resulting in tissue damage. The brain, due to its biochemical and physiological properties is especially sensitive to reactive oxygen species (ROS), which disrupt its functions and structure (Drewa et al., 1998).Vitamin C is an essential water-soluble vitamin that owes its effectiveness as an antioxidant to its oxido-reduction properties (Naidu, 2003). El Hossary et al. (2009) showed the protective effect of vitamins C and E on brain pathological changes induced by CPF in rats. Similarly, we have earlier demonstrated the effectiveness of vitamin C in mitigating short-term neurobehavioural changes induced by acute CPF exposure (Ambali et al., 2010c). The present study was aimed at evaluating the effect of VC on sensorimotor and cognitive changes induced by chronic CPF exposure in Wistar rats.

MATERIALS AND METHODS

Experimental animals and housing

Twenty young adult male Wistar rats weighing between 95g and 110g used for this study were obtained from the Laboratory Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The animals were allowed to acclimatize for at least two weeks in the laboratory prior to the commencement of the experiment. The animals were housed in plastic cages and fed on standard rat pellets while water was provided *ad libitum*. The experiment was conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Chemicals

Commercial grade CPF (20% EC, Termicot®, Sabero Organics, Gujarat limited, India), was prepared by reconstituting in soya oil (Grand Cereals and Oil Mills Ltd., Jos, Nigeria) to make 10% stock solution. Each tablet of ascorbic acid (100 mg; Med Vit C®, Dol-Med Laboratories Limited, Lagos, Nigeria) was dissolved in 1mL of distilled water to obtain 100 mg/mL suspension, just prior to its daily administration.

Animal treatment schedule

The rats were weighed and then assigned at random into

4 groups of 5 rats in each group. Group I (S/oil) served as the control and was given only soya oil (2mL/kg b.w.) while group II (VC) was dosed with vitamin C at 100 mg/kg b.w. (Ambali et al., 2010c, 2011). Group III (CPF) was administered with CPF only [10.6 mg/kg b.w. ~1/8th LD_{50} of 85 mg/kg b.w., as determined by Ambali (2009)]. Group IV (VC+CPF) was pretreated with vitamin C (100 mg/kg b.w.), and then dosed with CPF (10.6 mg/kg b.w.), 30 min later. The regimens were administered once daily by oral gavage for a period of 17 weeks. During this period, the animals were monitored for clinical signs and death. Similarly, at various intervals during the study period, the animals were evaluated for neurobehavioral parameters such as motor coordination, neuromuscular coordination, learning and memory. In order to avoid bias, the neurobehavioral parameters were evaluated by two trained observers blinded to the treatment schedule. At the end of the dosing schedule, the animals were sacrificed by jugular venesection and the brain removed and evaluated for oxidative stress parameters and level of AChE inhibition.

Evaluation of motor coordination

The assessment of motor coordination was performed using the beam walk performance task as described earlier (Ambali et al., 2010c) on day 0, weeks 8 and 16. Briefly, the rat from each group was allowed to walk across a wooden black beam of 106-cm length, beginning at 17.2-cm width and ending at 1.0-cm width. Periodic widths were marked on the side of the apparatus. On each side of the narrowing beam, there was a 1.8-cm step-down to a 3.0-cm area where subjects may step if necessary. As the subject walked across from the 17.2 cm to the 1.0 cm width, the width at which they stepped down was recorded by one rater on each side, and this was repeated twice during each trial session.

Assessment of neuromuscular coordination

The effect of treatments on neuromuscular coordination was assessed using the performance on inclined plane as was described earlier (Ambali et al., 2010c). Briefly, each rat was placed on an apparatus made with an angled rough wooden plank with thick foam pad at its bottom end. The plank was first raised to an inclination of 35°, and thereafter gradually increased stepwise by 5° until the subject could no longer stay and be situated horizontally on the plank for 3s, without sliding down. Angles were measured and marked on the apparatus beforehand, and were obtained by propping the plank on a vertical bar with several notches. The test was performed with the head of the rat first facing left and then right hand side of the experimenter. The highest angle at which each rat stayed and stood horizontally, and facing each direction was recorded. Two trials were performed at 2 min apart for each animal. This procedure was carried out on each animal from all the groups on day 0, week 8 and 16 of the study.

Effect of treatments on learning and short-term memory

The effect of treatments on learning task in rats was assessed 48h to the final termination of the study in week 17 using the step-down inhibitory avoidance learning task as described by Zhu et al. (2001). The apparatus used was an acrylic chamber 40 x 25 x 25 cm consisting of a floor made of parallel 2-mm-caliber stainless steel bars spaced 1 cm apart. An electric shock was delivered through the floor bars. A 2.5-cm-high, 8 x 25 cm wooden platform was placed on the left extreme of the chamber. Each animal was gently placed on the platform. Upon stepping down, the rat immediately received a single 1.5 amp foot shock through the floor bars. If the animal did not return to the platform, the foot shock was repeated every 5s. A rat was considered to have learned the avoidance task if it remained on the platform for more than 2 min. The number of foot shocks was recorded as an index of learning acquisition.

Memory was assessed in individual rat from each group 24h after the assessment of learning using the same. In this test, each rat was again placed gently on the platform and the time an animal remained on the platform was recorded as an index of memory retention. Staying on the platform for 2 min was counted as maximum memory retention (ceiling response).

Effect of treatments on brain lipoperoxidation

The level of thiobarbituric acid reactive substance, malonaldehyde (MDA) as an index of lipid peroxidation was evaluated on the brain sample using the method of Draper and Hadley (1990) as modified (Freitas et al., 2005). The principle of the method was based on spectrophotometric measurement of the colour developed during reaction of thiobarbituric acid (TBA) with malonadehyde (MDA). The MDA concentration in each sample was calculated by the absorbance coefficient of MDA-TBA complex 1.56 x 10^5/cm/M and expressed as nmol/mg of tissue protein. The concentration of protein in the brain homogenates was evaluated using the Lowry method (Lowry et al., 1951).

Effect of treatments on brain superoxide dismutase activity

Superoxide dismutase (SOD) activity was evaluated using NWLSS^{TM} superoxide dismutase activity assay kit

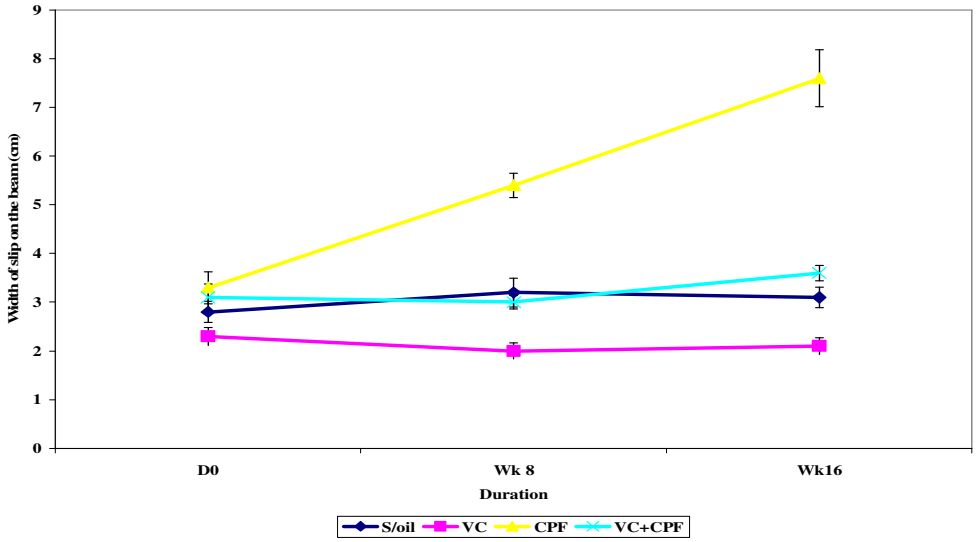

Figure 1. Effects of soya oil, vitamin C and/or chlorpyrifos on the dynamics of beam walk performance in Wistar rats.

(Northwest Life Science Specialities, Vancouver, WA 98662) as stated by the manufacturer.

Effect of treatments on brain catalase activity

Catalase (CAT) activity was evaluated using NWLSS^{TM} catalase activity assay kit (Northwest Life Science Specialities, LLC, Vancouver, WA 98662) as stated by the manufacturer.

Effect of treatments on brain acetylcholinesterase activity

Acetylcholinesterase activity was evaluated using the method of Ellman et al. (1961) with acetylthiocholine iodide as a substrate. Briefly, the whole brain of each animal was homogenized in a cold (0–4 ℃) 20 m*M* phosphate buffer saline (PBS) incubated with 0.01M 5,5-dithio-bis(2-nitrobenzoic acid) in 0.1 *M* PBS, pH 7.0. Incubations were allowed to proceed at room temperature for 10 min. Then, acetylthiocholine iodide (0.075 *M* in 0.1 M PBS, pH 8.0) was added to each tube, and absorbance at 412 nm was measured continuously for 30 min using a UV spectrophotometer (T80^{+} UV/VIS spectrometer®, PG Instruments Ltd, Liicestershire, LE 175BE, United Kingdom). AChE activity expressed as IU/g tissue was calculated based on the rate of color change per minute.

Statistical analysis

Data were expressed as mean ± standard error of mean. Beam walk and incline plane performances were analyzed using repeated one-way analysis of variance followed by Tukey's test. The values recorded in learning and memory tests were analyzed using the Kruskal-Wallis one way analysis of variance on ranks followed by Dunn's multiple comparison test. The MDA, AChE, CAT and SOD were analyzed using one-way analysis of variance followed by Tukey's post hoc test. Values of $P < 0.05$ were considered significant.

RESULTS

Clinical signs

There were no apparent clinical signs observed in the S/oil, VC and VC+CPF groups, while lacrimation, congested ocular mucous membranes and intermittent tremors were recorded for rats in the CPF group.

Motor coordination

The width at which rats in the CPF group slipped off the beam was significantly higher ($p<0.01$) at either week 8 or 16 when compared to the value recorded at day 0 (Figure 1). The beam walk slipping width was significantly higher at week 16 compared to week 8 in the CPF group. There was no significant ($p>0.05$) change in the beam walk slipping width of rats in the S/oil, VC or VC+CPF group at day 0 when compared to either week 8 or 16. However, the beam walk slipping width for rats in the VC+CPF group was significantly higher ($P<0.05$) at week 16 when compared to week 8.

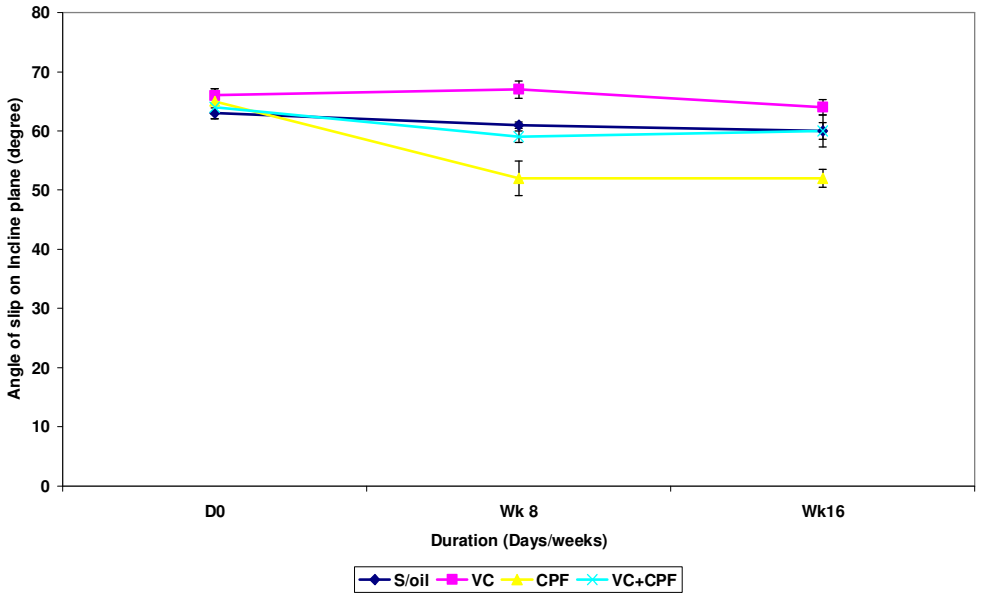

Figure 2. Effect of chronic exposure of Wistar rats to soya oil, chlorpyrifos and/or vitamin C on dynamics of inclined plane performance.

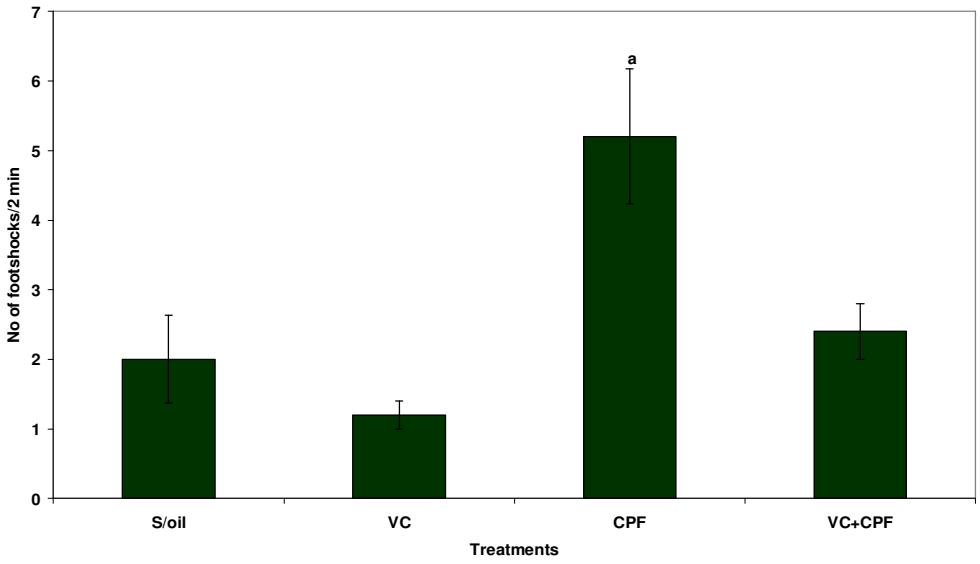

Figure 3. Effect of chronic administration of soya oil, chlorpyrifos and/or vitamin C on learning acquisition in Wistar rats. [a]$p<0.01$ versus vitamin C group. Values are mean±SEM of 5 animals per group.

Neuromuscular coordination

The effect of treatments on neuromuscular coordination as reflected by dynamics of performance on the inclined plane is shown in Figure 2. The angle at which rats in the CPF group slipped off the apparatus at day 0 was significantly higher ($p<0.01$) compared to those obtained by weeks 8 and 16. The angle at which rats in the CPF group slipped off the incline plane apparatus by week 16 was not significantly different ($p>0.05$) from those recorded by week 8. There was no significant ($p>0.05$) change in the angle at which rats in either the S/oil, VC or VC+CPF group slipped off the plane at day 0 compared to either week 8 or 16.

Learning acquisition

The number of footshocks applied to rats in the CPF group was significantly higher ($p<0.01$) compared to VC

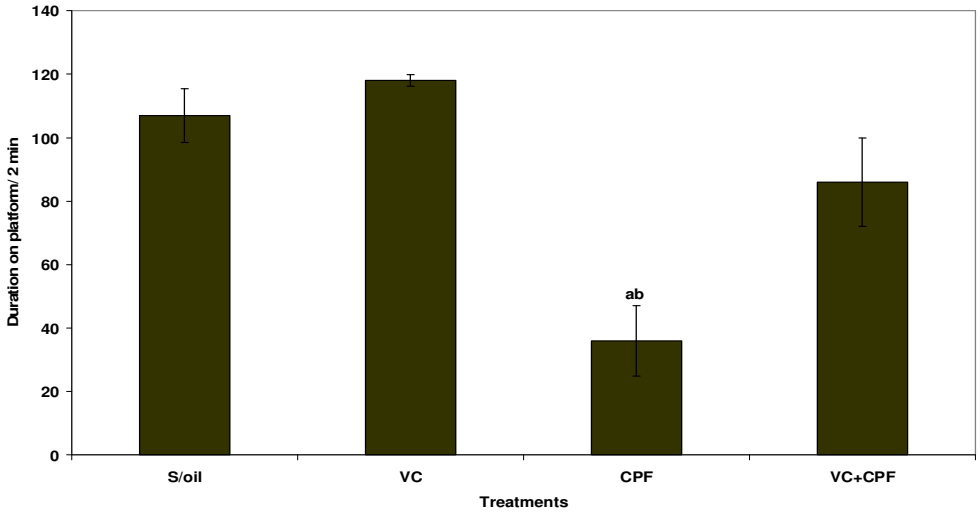

Figure 4. Effect of chronic administration of soya oil, chlorpyrifos and/or vitamin C on short-term memory in Wistar rats. [a]p<0.01 versus soya oil group; [b]p<0.01 versus vitamin C group. Values are mean±SEM of 5 animals per group.

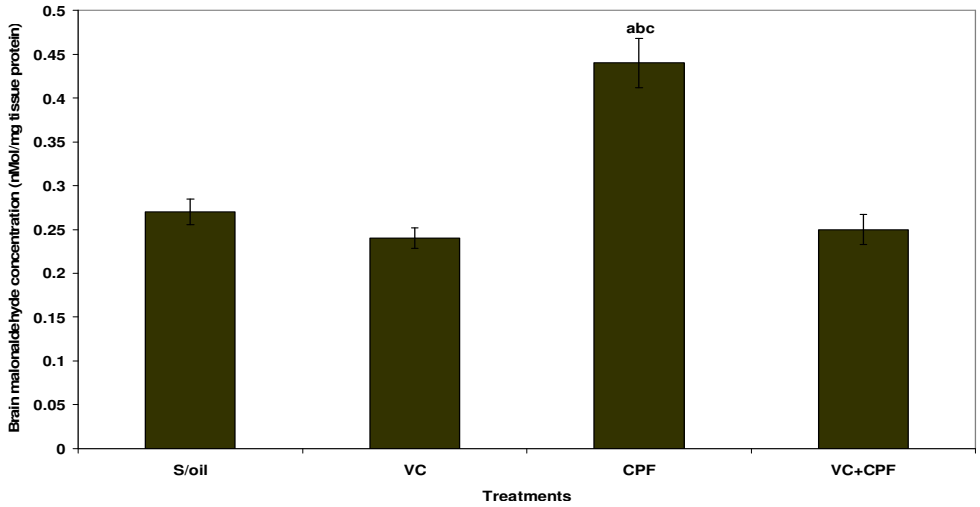

Figure 5. Effect of chronic exposure to soya oil, chlorpyrifos and/or vitamin C on brain malonaldehyde concentration in Wistar rats. [a]p<0.01 versus soya oil group; [b]p<0.01 versus vitamin C group; [c]p<0.01 versus vitamin C+chlorpyrifos group

group. Although there was no significant change (p>0.05), the number of footshocks applied to the rats in the CPF group, however, increased by 62% and 53%, respectively, when compared to S/oil and VC+CPF groups (Figure 3).

Short-term memory

The duration on the platform by rats in the CPF group was significantly lower (p<0.05) compared to either those in the S/oil or VC group. There was no significant change (p>0.05) in the duration on the platform for rats in the VC+CPF group compared to either those in the S/oil, CPF or VC group. However, the duration on the platform of rats in the VC+CPF group was 57% longer than those recorded in the CPF group (Figure 4).

Brain malonaldehyde concentration

The concentration of brain MDA in the CPF group was significantly (p<0.01) higher compared to those recorded in either the S/oil, VC or VC+CPF group. There was no significant (p<0.05) change in the MDA concentration in the VC+CPF group compared to either those in the S/oil or VC group (Figure 5).

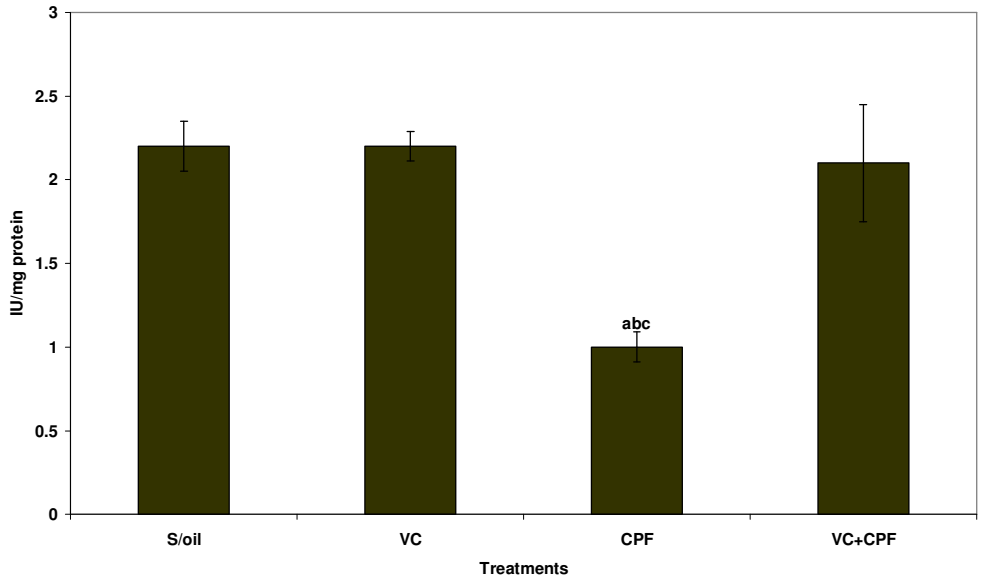

Figure 6. Effect of chronic exposure of Wistar rats to soya oil, chlorpyrifos and/or vitamin C on brain superoxide dismutase activity. [a]p<0.05 versus soya oil group; [b]p<0.05 versus vitamin C group; [c]p<0.05 versus vitamin C+chlorpyrifos group. Values are mean±SEM of 5 animals per group.

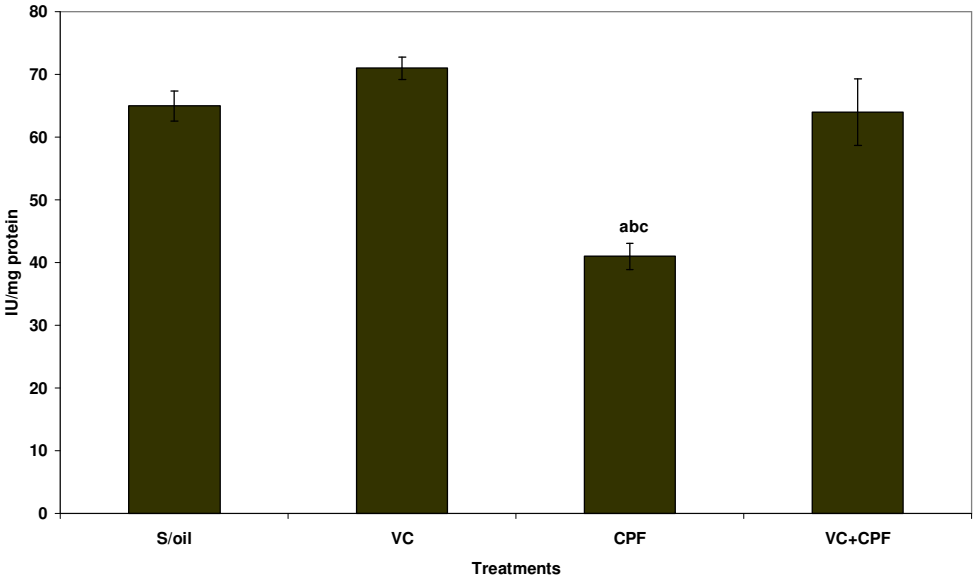

Figure 7. Effect of chronic administration of soya oil, chlorpyrifos and/or vitamin C on brain catalase activity in Wistar rats. [a]p<0.01 versus soya oil group; [b]p<0.01 versus vitamin C group; [c]p<0.01 versus vitamin C+chlorpyrifos group. Values are mean±SEM of 5 animals per group.

Brain superoxide dismutase activity

The effect of treatments on brain SOD activity is shown in Figure 6. The SOD activity in the CPF group was significantly ($p<0.05$) lower than those recorded in either the S/oil, VC or VC+CPF group. There was no significant ($p>0.05$) change in the SOD activity in the VC+CPF group when compared to either the S/oil or VC group.

Brain catalase activity

The brain CAT activity in the CPF group was significantly ($p<0.01$) lower in the CPF group compared to either the S/oil, VC or VC+CPF group. There was no significant ($p>0.05$) change in CAT activity in the VC+CPF group compared to either the S/oil or VC group (Figure 7).

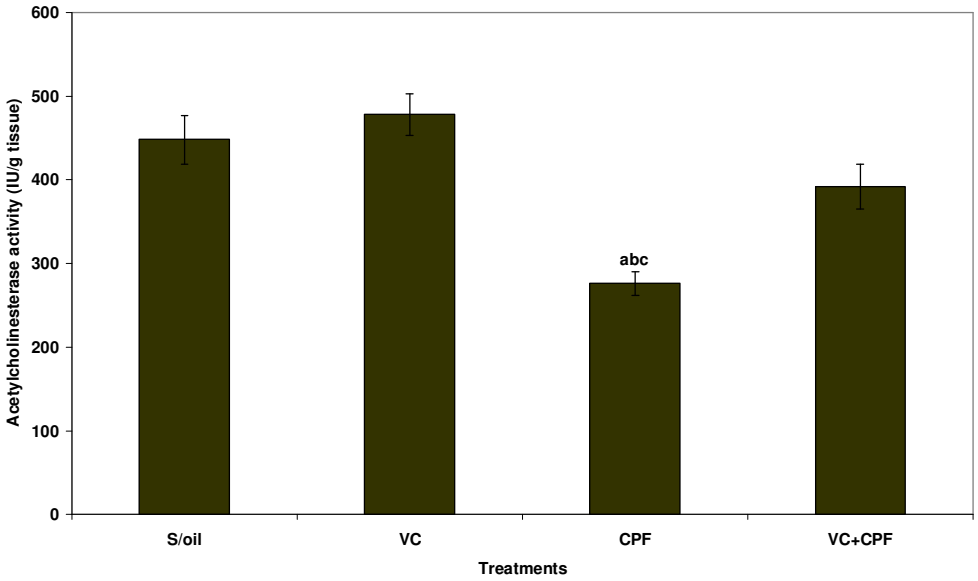

Figure 8. Effect of chronic administration of soya oil, chlorpyrifos and/or vitamin C on brain acetylcholinesterase activity in Wistar rats. [a]p<0.01 versus soya oil group; [b]p<0.01 versus vitamin C group; [c]p<0.05 versus vitamin C+chlorpyrifos group. Values are mean±SEM of 5 animals per group.

Brain acetylcholinesterase activity

The brain AChE activity was significantly lower in the CPF group compared to either the S/oil (p<0.01), VC (p<0.01) or VC+CPF (p<0.05) group. No significant change (p>0.05) was recorded in the brain AChE activity in the VC+CPF group compared to either the S/oil or VC group (Figure 8).

DISCUSSION

The present study has shown that chronic CPF exposure increased the level of MDA, indicating increased brain lipoperoxidation. Earlier studies have shown increased lipoperoxidation in the liver (Goel et al., 2005; Ambali, 2009) and erythrocytes (Ambali et al. 2010a,b, 2011) of rats following CPF exposure. The brain is very susceptible to oxidative stress, and in case of accelerated oxidative challenge due to CPF exposure, it is one of the most vulnerable organs. Chronic CPF exposure similarly caused significant inhibition of the activities of brain antioxidant enzymes, SOD and CAT. Both are the two basic subcellular defense of antioxidant enzymes that counteracts free radicals produced during xenobiotic exposure (Halliwell, 1994). SOD is a first line antioxidant enzyme that dismutate $O_2^{\bullet-}$ to H_2O_2, which is decomposed by CAT to H_2O (Fridovich, 1975). The decline in the activity of the antioxidant enzymes following chronic CPF exposure in the present study may be due to downregulation in the synthesis of antioxidant enzymes due to persistent toxicant insult (Irshad and Chaudhuri, 2002). The decrease in brain CAT activity may also be due to its inactivation by $O_2^{\bullet-}$ (Kono and Fridovich, 1982). The implication of the reduced activities of the two enzymes is the exacerbation of the formation of the more potent tissue damaging $OH^{\bullet}$.

Vitamin C pretreatment was however, shown to have decreased the brain MDA concentration and increased brain SOD and CAT activities indicating the ability of the antioxidant to ameliorate CPF-induced oxidative damage. VC by its antioxidant effect was able to quench the free radicals that are generated due to CPF exposure, resulting in the preservation of the activities of SOD and CAT. Although, VC is a water soluble free radical scavenger in the extracellular fluid, the brain is one of the organ with the highest ascorbate concentration (Hornig, 1975) where it acts as an intracellular antioxidant in the neurons and as an antioxidant in the brain extracellular microenvironment (Rice, 2000; Harrison and May, 2009). Therefore, VC constitutes an important neuroprotective constituent (Naseer et al., 2009). Although water soluble, VC finds its way into the brain in significant quantity via different processes, mostly involving active stereospecific sodium-dependent transport at the choroid plexus (Spector and Lorenzo, 1973), carrier-mediated uptake and by simple diffusion across brain capillaries at the blood-brain barriers (Rice, 2000)

Although the CPF was administered at low dose, it was still able to reduce AChE activity significantly, apparently due to the cumulative nature of the exposure and the highly lipophilic nature of the toxicant. The reduced AChE activity in the CPF group resulted in some form of cholinergic toxicity, which apparently played an

important role in the clinical and behavioral toxicity observed in the group. The antioxidant vitamin was however able to restore the AChE activity. Vitamins C and E have been shown to restore AChE activity inhibited by OP insecticide (Yavuz et al., 2004; Ambali et al., 2010c). The reduced oxidative changes recorded in the group pretreated with VC due to its antioxidant effect may be partly responsible for the improvement in AChE activity. Oxidative stress is known to affect the activities of various membrane-bound enzymes, including AChE (Mehta et al., 2005) via their direct attack by free radicals or peroxidation of the membrane lipids in which they are embedded (Souzal et al., 2010). Therefore, VC, as a free radical scavenger, trapped the reactive oxygen species thereby interrupting the chains of oxidative reactions that alter AChE activity. This demonstrates a relationship between free radical and AChE inhibition (Tsakiris et al., 2000).

The lacrimation and intermittent tremors observed in the group exposed to CPF only were part of the cholinergic signs associated with OP insecticides (Eaton et al., 2008*)*. These cholinergic signs were due to inhibition of AChE by CPF, resulting in accumulation of ACh in the muscarinic and nicotinic cholinergic receptors. However, the rats pretreated with VC did not manifest any overt clinical sign, indicating that the vitamin protected the neurons from AChE inhibition. This finding agreed with our earlier studies that showed that vitamin C mitigated cholinergic signs induced by CPF (Ambali et al., 2007, 2010a, 2011). The improvement in the brain AChE activity recorded in the group pretreated with VC in the present study may have contributed to the mitigation of cholinergic toxicity in this group. VC had earlier been shown in our laboratory to reactivate AChE inhibited by acute CPF exposure in rats (Ambali et al., 2010c). Similar result has been reported following exposure to another OP compound, methidathion, in rats (Yavuz et al., 2004). Besides, VC has also been shown to improve paraoxonase activity (Jarvik et al., 2002), thereby aiding the detoxification of the OP compound (Shih et al., 1998).

The significant increase in the width at which rats in the CPF group slipped off the beam was an indication of reduction in beam-walk length, thus impairment of motor coordination. The depreciation in beam-walk length in the CPF group progressed with increased duration of exposure, with much more deficit observed in week 16 than in week 8. We have earlier shown that subacute CPF exposure impaired motor coordination in Wistar rats (Ambali et al., 2012). Abou-Donia et al. (2002) observed similar results following repeated exposure of rats to sarin. Beam-walking performance, an integrated form of behavior that requires pertinent level of consciousness, memory, sensorimotor and cortical functions is mediated by the cortical area (Abou-Donia et al. 2001). Therefore, the deficit in beam-walk length in the CPF group may be due to cortical injury (Abou-Donia et al., 2001), perhaps partly due to oxidative damage. The brain is very susceptible to oxidative stress, because it harbors large amount of oxygen in a relatively small mass, contains significant quantity of pro-oxidant metal (Fe) and peroxidizable lipids, and has fewer antioxidant molecules than other tissues (Halliwell and Gutteridge, 1999). The increased lipoperoxidation and deficit in antioxidant enzymes in the brain of rats exposed to CPF only in the present study may have played some role in the motor coordination impairment in this group.

Pretreatment with VC improved the beam-walk length of the rats significantly by decreasing the width at which they slipped off the beam. This indicates restoration of motor coordination impaired by chronic CPF exposure. However, the fact that the width of slip was significantly higher in week 16 compared to week 8 shows that VC may not be entirely protective of motor coordination impairments, especially in prolonged exposure. This shows that oxidative stress may not be entirely responsible for the beam walk deficit induced by prolonged CPF exposure. However, the result clearly demonstrates that oxidative damage to some portions of the brain, particularly the cortical areas may be partly responsible for the beam walk deficit in the CPF group. Hence, the improvement in beam-walk length observed in group pretreated with VC especially by week 8 may be partly due to a decrease in the level of CPF-induced oxidative damage. Besides, VC plays an essential role in the formation of norepinephrine (Iqbal et al., 2004) that plays a role in beam walk deficits induced by cortical injury (Boyeson et al., 1992; Goldstein, 1995).

The poor performance of rats exposed to CPF only on inclined plane demonstrates that the OP insecticide impaired sensorimotor performance and neuromuscular coordination. The deficit in the neuromuscular coordination increased with the duration of CPF exposure to a certain extent since there was an apparent decrease in the inclined plane performance of the rats in week 8 compared to those recorded in day 0. However, the fact that there was no apparent change in the incline plane performance of rats in the CPF group in week 16 compared to week 8 may indicate the development of some form of tolerance to the insecticide, a situation which has been reported following subchronic and chronic OP exposure (Russell and Overstreet, 1987; Swamy et al., 1993). Behavioral tolerance in the face of sustained AChE inhibition following prolonged exposure to OP compound has been attributed to early recovery of pseudocholinesterase activity (Chetan et al., 2009). Results similar to those obtained in the present study had earlier been reported in Wistar rats following subacute CPF exposure (Ambali et al., 20012). Abou-Donia et al. (2002) also showed that sarin, an OP warfare agent impaired the inclined plane performance in rats. Pretreatment with VC was shown in the present study to ameliorate the poor performance on inclined plane induced by repeated exposure to CPF. This further demonstrates the involvement of brain oxidative stress in

the mechanism of CPF-induced impairment of neuromuscular coordination in Wistar rats.

Chronic repeated CPF exposure has also been shown to apparently impair learning and significantly reduce short-term memory in the present study. This result agreed with many other studies, which demonstrated that repeated low-level exposure to CPF impair cognition in rats (Stone et al., 2000; Moser et al., 2005; Prendergast et al., 2007; Ambali et al., 2012). In addition, studies in humans have shown persistent cognitive deficits in farmers and pesticide applicators repeatedly exposed to OPs but are symptom-free (Steenland et al. 2000; Dick et al. 2001). The impairment of cognition observed in rats dosed with CPF only may be due to alteration in ACh metabolism caused by AChE inhibition. Many studies have linked central cholinergic system to synaptic plasticity, learning and memory processes (Baskerville et al., 1997; Sachdev et al., 1998). It is believed that OP compounds play a role in memory loss by producing cholinergic dysfunction at the level of the synapse (Carr and Chambers, 1991). Low-level CPF exposure has been shown to induce protracted deficits in information processing and cognitive functions, consequent from functional changes in brain cholinergic pathways resulting from alteration in bidirectional fast axonal transport (Terry et al., 2007). Since ACh has been demonstrated to be involved in cognition, agents such as OPs which alter ACh metabolism may perturb cognitive processes.

However, other studies have shown the ability of CPF to induce cytotoxic effects directly on the hippocampal cells via the induction of apoptosis, irrespective of its effect on AChE (Terry et al., 2003). We have earlier demonstrated apoptotic damage to the neuronal cells in brain of rats following chronic CPF exposure (Ambali and Ayo, 2011). This may have played some roles in the CPF-induced cognitive deficit in the present study since apoptosis has been described as the toxic end-point of CPF neurotoxicity in the brain (Caughlan et al., 2004). Apoptosis induces structural changes in the brain resulting in functional deficits, including those involved in memory and learning task (Caughlan et al., 2004). Free radicals have been implicated in apoptotic death of cells (Corcoran et al., 1994; McConkey et al., 1994) and may be involved in the cognitive impairments recorded in the CPF group. Neuronal degeneration, which we have earlier demonstrated following chronic CPF exposure in rats (Ambali and Ayo, 2011) is mechanically related to neuronal oxidative stress (Gupta et al., 2007) and may be central to cognitive dysfunction observed in the group exposed to CPF only

The apparent improvement in cognitive performance following vitamin C pretreatment may have been partly due to restoration of AChE activity thereby reducing the cholinergic alterations, which play a significant role in synaptic plasticity, memory and learning. Furthermore, the reduced ROS generation in vitamin C pretreated group may have contributed to the reduction of AChE inhibition. The reduced oxidative damage in the VC pretreated group may have contributed to the decline in ROS-induced apoptotic damage. VC has been shown to reduce neuronal apoptosis induced by caspase-3 in alcohol-induced neuronal death (Naseer et al., 2009).

The non antioxidant role of VC may have complemented its antioxidant effect in the mitigation of CPF-induced clinical and behavioral toxicity observed in the present study. VC is a neuromodulator of both dopamine and glutamate-mediated neurotransmission (Grünewald 1993) and an essential cofactor in the synthesis of noradrenaline (Diliberto et al., 1987) and many neuropeptides (Glembotski, 1987). In addition, VC promotes myelin formation by Schwann cells by enabling these cells to assemble basal lamina (Eldridge et al., 1987). These may have complemented its protective effect in CPF-induced neurotoxicity.

In conclusion, the present study has shown that clinical, sensorimotor and cognitive deficits induced by chronic CPF exposure in Wistar rats are partly mediated by oxidative stress. In addition, VC has been shown to mitigate these clinical and neurobehavioral changes partly due to its neuroprotective role predominantly arising from its antioxidant and AChE restoration properties. Therefore, VC may protect individuals exposed to frequent low-dose CPF either from occupational or environmental source from the OP-induced neurobehavioral changes.

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