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Ameliorative Effect of Vitamin E on Sensorimotor and Cognitive Changes Induced by Chronic Chlorpyrifos Exposure in Wistar Rats

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1. Introduction

The use of pesticides is inevitable in contemporary world because of their role in the improvement of food production through increase in crop yields and quality, reduction of farm labour requirements hence lowering cost of production, and improving public health through control of vector and vector-borne diseases (Weiss et al., 2004). Despite all these benefits, pesticides constitute menace to the health of man, animals and even the environment. This is because they are poorly selective and are toxic to non-target species, including humans. The segments of the population that are at the greatest risk of exposure are those that are occupationally exposed, such as agricultural workers. Despite the strict measures put in place concerning its commercialization and use, pesticides sales has increased in recent years (Carlock et al., 1999). The World Health Organization (WHO) estimated that about 3 million cases of acute intoxication and 220,000 deaths are attributable to pesticides each year with majority of these cases occurring in less developed countries (He, 2000; Clegg & van Gemert, 1999), particularly in Africa, Asia, Central America, and South America (Pancetti et al., 2007). Although many pesticides cause neurotoxicity, insecticides are the most acutely neurotoxic to humans and other non-target species compared to other pesticides (Costa et al., 2008). Association between acute exposure to pesticides and neurotoxicity is well known (Lotti, 2000) but the potential effects of chronic low-level exposure are less well established (Alavanja et al., 2004; Ambali et al., 2010a; Ambali & Aliyu, 2012).

Organophosphate (OP) compounds are one of the most widely used constituting about 50% global insecticide use (Casida & Quistad, 2004). 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 & 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. The principal mode of insecticidal action of OPs relates to phosphorylation and subsequent inactivation of the esteratic sites of the acetylcholinesterase (AChE) enzyme. The classical role of AChE is to hydrolyze the neurotransmitter acetylcholine (ACh), effectively clearing it from the neuronal synapse and terminating impulse conduction (Farag et al., 2010). Inactivation of AChE results in the accumulation of ACh in the neuronal synapses in the central and peripheral nervous system, thereby overstimulating the nicotinic, muscarinic and central cholinergic receptors with consequent neurotoxicity. Thus, the acute neurotoxic effect of OP results in muscarinic, nicotinic and central cholinergic symptoms (Abou-Donia, 1992). However, toxicity has been reported at doses below the threshold required for inhibition of AChE (Pope, 1999; Slotkin, 2004, 2005) prompting search for other mechanisms. The induction of oxidative stress as one of the other molecular mechanisms involved in OP-induced neurotoxicity has received tremendous attention in recent years (Gultekin et al., 2007; Prendergast et al., 2007; El-Hossary et al., 2009; Ambali et al., 2010a, Ambali & Ayo, 2011a, 2011b; Ambali & Aliyu, 2012). Indeed, the enhanced production of reactive oxygen species (ROS) by pesticides has been used to explain the multiple types of responses associated with its toxic exposure (Bagchi et al., 1995; Verma et al., 2007).

Chlorpyrifos (*O,O*-diethyl-*O*-[3,5,6-trichloro-2-pyridyl] phosphorothioate) is a chlorinated OP insecticide that exhibit a broad spectrum of activity against arthropod pests of plants, animals, and humans, and has wide applications in both agricultural and commercial pest control (Rack, 1993). It is one of the most widely used insecticides and is applied about 20 million times per year in US to houses and lawns (Kingston et al., 1999) with 82% of adults having detectable levels of the 3,5,6-trichloro-2-pyridinol, the metabolite of CPF in their urine (Hill et al., 1995). However, the United States Environmental Protection Agency in 2000 placed ban on some its residential uses in 2000 because of the danger posed to children's health. However, CPF is still widely used as its residues have been detected in citrus fruits in some parts of the world (Iwasaki et al., 2007). Studies have shown that CPF induces neurobehavioural alterations following acute (Cañadas et al., 2005; Ambali et al., 2010a, Ambali & Aliyu, 2012) and repeated low-dose (Stamper et al., 1988; Sanchez-Santed et al., 2004; Ambali & Ayo, 2011a, 2011b) exposure. Similarly, CPF is a developmental neurotoxicant (Qiao et al., 2003; Dietrich et al., 2005; Colborn, 2006; Slotkin et al., 2006;) impairing children mental and behavioral health (Lizardi et al., 2008). Although, CPF like the other OP compounds phosphorylates and subsequently inactivate AChE, neurobehavioural and cognitive deficits have however been observed following repeated low-dose CPF exposure that cannot be attributed to the usual AChE inhibition and muscarinic receptor binding (Pope et al., 1992; Chakraborti et al., 1993; Saulsbury et al., 2009). Earlier studies have shown the involvement of oxidative stress in the neurotoxicity induced by CPF exposure (Gultekin et al., 2007; Ambali et al., 2010a; Ambali & Aliyu, 2012; Ambali and Ayo, 2011a, 2011b).

Oxidative stress, defined as a disruption of the prooxidant-antioxidant balance in favor of the former causes damage to the body tissue (Sies, 1991). Oxidative stress results from an increase in ROS, impairment of antioxidant defense system or insufficient capacity to repair oxidative damage (Halliwell, 1994; Aly et al., 2010). Damage induced by ROS which alters cellular macromolecules such as membrane lipids, DNA, and proteins results in impaired cell functions through changes in intracellular calcium or pH, and consequently leads to cell

death (Kehrer, 1993; Sally et al., 2003). The body is however endowed with cellular defence systems to combat the menace posed by the oxidants to the body. These defensive systems are accomplished by the activities of both the enzymatic and non-enzymatic antioxidants which mitigate the toxic effect of oxidants. However, under increased ROS production, the antioxidant cellular defensive systems are overwhelmed, resulting in oxidative stress. Under this type of condition, exogenous supplementation of antioxidants becomes imperative to minimise tissue damage.

Vitamin E is nature's major lipid soluble chain breaking antioxidant that protects biological membranes and lipoproteins from oxidative stress (Osfor et al., 2010). The main biological function of vitamin E is its direct influence on cellular responses to oxidative stress through modulation of signal transduction pathway (Hsu & Guo, 2002). Vitamin E primarily scavenges peroxy radicals and is a major inhibitor of the free radical chain reaction of lipid peroxidation (Maxwell, 1995; Halliwell & Gutteridge, 1999). We have earlier demonstrated the mitigating effect of vitamin E on short-term neurobehavioural changes induced by acute CPF exposure (Ambali & Aliyu, 2012). The present study was therefore aimed at evaluating the ameliorative effect of vitamin E on sensorimotor and cognitive changes induced by chronic CPF exposure in Wistar rats.

2. Materials and methods

2.1 Experimental animals and housing

Twenty 10 week old male Wistar rats (104 ± 4.2) 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 housed in plastic cages and allowed to acclimatize for at least two weeks in the laboratory prior to the commencement of the experiment. They were fed on standard rat pellets and water was provided *ad libitum*.

2.2 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. Vitamin E (100 mg/capsule; Pharco Pharmaceuticals, Egypt) was reconstituted in soya oil (100% v/v) prior to daily use.

2.3 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 (VE) was dosed with vitamin E [75 mg/kg b.w. (Ambali et al., 2010b)]. Group III (CPF) was administered with CPF only [10.6 mg/kg b.w. $\sim 1/8^{\text{th}}$ LD₅₀ of 85 mg/kg b.w., as determined by Ambali (2009)]. Group IV (VE+CPF) was pretreated with vitamin E (75 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. Furthermore, at various intervals during the study period, the animals were evaluated for neurobehavioural parameters measuring motor coordination, neuromuscular coordination, and motor strength, efficiency of locomotion, learning and memory using the appropriate neurobehavioural devices. In order to avoid bias, the neurobehavioural parameters were evaluated by two trained observers blinded to the treatment schedules. At the end of the dosing period,

each of the animals was sacrificed by jugular venesection and the brain dissected, removed and evaluated for the levels of oxidative stress parameters and AChE inhibition. The experiment was conducted with the permission of the Animals Research Ethics Committee of the Ahmadu Bello University, Zaria, Nigeria and in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.4 Evaluation of the effect of treatments on motor coordination

The assessment of motor coordination was performed using the beam walk performance task as described in an earlier study (Ambali et al., 2010a) on day 0, weeks 8 and 16. Briefly, each of the rats 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.

2.5 Evaluation of the effect of treatments on motor strength

The forepaw grip time was used to evaluate the motor strength of the rats, as described by Abou-Donia et al. (2001). This was conducted by having each of the rats hung down from a 5 mm diameter wooden dowel gripped with both forepaws. The time spent by each rat before releasing their grips was recorded in seconds. This parameter was evaluated on day 0, weeks 8 and 16.

2.6 Effect of treatments on neuromuscular coordination

The effect of treatments on neuromuscular coordination was assessed using the performance on incline plane as was described earlier (Ambali et al., 2010a). 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, weeks 8 and 16 of the study.

2.7 Evaluation of the effect of treatments on efficiency of locomotion

The ladder walk was used to assess the efficiency of locomotion as described by Ambali and Aliyu (2012). Briefly, each rat was encouraged to walk across a black wooden ladder (106 cm x 17 cm) with 0.8-cm diameter rungs, and 2.5-cm spaces between them. The number of times the rat missed a rung was counted by one rater on each side. The performance on ladder walk was evaluated on Day 0, weeks 3, 7 and 11. Two trials were performed for each testing session.

2.8 Assessment of the effect of treatments on learning

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 rat 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.

2.9 Assessment of the effect of treatments on short-term memory

Short-term memory was assessed in individual rat from each group using the step-down avoidance inhibitory task as described by Zhu et al. (2001) 24h after the assessment of learning. The apparatus used was the same used earlier for the assessment of learning. 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).

2.10 Brain tissue preparation

The whole brain tissue was carefully dissected and a known weight of the brain sample from each animal was homogenized in a known volume of ice cold phosphate buffer to obtain a 10% homogenate. This was then centrifuged at $3000 \times g$ for 10 min to obtain the supernatant. The supernatant was then used to assess the levels of protein, malonaldehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and AChE in the brain sample.

2.11 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 & 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 $1.56 \times 10^5/\text{cm}/\text{M}$ 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).

2.12 Evaluation of the effect of treatments on brain superoxide dismutase activity

Superoxide dismutase activity was evaluated using NWLSS™ superoxide dismutase activity assay kit (Northwest Life Science Specialities, Vancouver, WA 98662) as stated by the manufacturer and was expressed as mMol/mg tissue protein.

2.13 Evaluation of the effect of treatments on brain catalase activity

Catalase activity was evaluated using NWLSS™ catalase activity assay kit (Northwest Life Science Specialities, LLC, Vancouver, WA 98662) as stated by the manufacturer and was expressed as mMol/mg tissue protein.

2.14 Evaluation of the 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 °C) 20 mM 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, Lliestershire, LE 175BE, United Kingdom). AChE activity was expressed as IU/g tissue.

2.15 Statistical analysis

Data were expressed as mean $\pm$ standard error of mean. Data obtained from the sensorimotor assessment were analyzed using repeated one-way analysis of variance followed by Tukey's posthoc test. The cognitive and biochemical parameters were analyzed using one-way analysis of variance followed by Tukey's posthoc test. Values of $P < 0.05$ were considered significant.

3. Results

3.1 Effect of treatments on clinical signs

There was no clinical manifestation recorded in the S/oil, VE and VE+CPF groups, while lacrimation, congested ocular mucous membranes and intermittent tremors were observed in the CPF group.

3.2 Effect of treatments on beam walk performance

There was no significant change ($P > 0.05$) in the dynamics of beam walk performance in the S/oil group throughout the period of the study. There was a progressive decrease in the width at which VE group slipped off the beam (increase in beam walk length) throughout the study period. Although no significant change ($P > 0.05$) was recorded in week 8 compared to day 0 or week 16, a significant decrease ($P < 0.05$) in the width at which the VE group slipped off the beam in week 16 compared to that of day 0. There was a significant increase ($P < 0.01$) in the width of slip off the beam (decrease in beam walk length) in the CPF group at weeks 8 and 16 when compared to that of day 0, and between week 16 and that recorded in week 8. There was no significant change ($P > 0.05$) in the width at which VE+CPF group slipped off the beam at week 8 when compared to that recorded on day 0 or week 16 but a significant increase ($P < 0.01$) was recorded at week 16 compared to that of day 0.

There was no significant change ($P > 0.05$) in the width at which animals in all the groups slipped off the beam at day 0. At week 8, there was a significant increase ($P < 0.01$) in the width at which the CPF group slipped off the beam compared to that of S/oil, VE or VE+CPF group. Similarly, there was a significant increase ($P > 0.05$) in the width of slip in the VE+CPF group compare to that of VE group but no significant change ($P > 0.05$) in the S/oil group compared to that of VE or VE+CPF group. At week 16, there was a significant increase ($P < 0.01$) in the width of slip off the beam in the CPF group compared to the other groups but no significant change ($P > 0.05$) in the S/oil group when compared to that of VE or VE+CPF group, and between VE group and that recorded in the VE+CPF group (Fig. 1).

Fig. 1. Effect of chronic administration of soya oil, vitamin E and/or chlorpyrifos on the dynamic of beam walk performance in Wistar rats.

3.3 Effect of treatments on grip time

There was no significant change ($P>0.05$) in the grip time in the S/oil and VE groups throughout the study period. There was a significant increase ($P<0.01$) in the grip time of CPF and VE+CPF groups at day 0 compared to that of week 8 or 16, but not between week 8 and that of week 16. At day 0, there was no significant change ($P>0.05$) in the grip time of rats in between the groups. At week 8, there was a significant decrease ($P<0.01$) in the grip time of CPF group compared to that in the S/oil and VE groups, but not that of VE+CPF group. There was a significant decrease ($P<0.05$) in the grip time in the VE+CPF group compared to that in S/oil or VE group. There was no significant change ($P>0.05$) in the grip time in the VE group compared to that in S/oil group. At week 16, there was a significant decrease ($P<0.01$) in the grip time in the CPF group compared to that in S/oil or VE group but no significant change ($P<0.05$) compared to that in VE+CPF group. There was no significant change ($P>0.05$) in the grip time in the VE+CPF group compared to that in S/oil or VE group. Similarly, there was no significant change ($P>0.05$) in the grip time of S/oil group compared to that in VE group (Fig. 2).

3.4 Effect of treatments on incline plane performance

There was no significant change ($P>0.05$) in the angle at which the S/oil and VE groups slipped off the incline plane throughout the study period. There was a significant decrease ($P<0.05$) in the angle at which the CPF group slipped off the incline plane at weeks 8 and 16, respectively, compared to that of day 0 but no significant change ($P>0.05$) at week 8 relative to that recorded in week 16. There was a significant decrease ($P<0.01$) in the angle at which VE+CPF group slipped off the incline plane at week 16 compared to that of day 0 but no significant change ($P>0.05$) at week 8 relative to that recorded in day 0 or week 16.

Fig. 2. Effect of chronic administration of soya oil, vitamin E and/or chlorpyrifos on the dynamic of grip time in Wistar rats.

At day 0, there was no significant change ($P>0.05$) in the angle of slip off the incline plane in between the groups. At week 8, there was a significant decrease in the angle of slip off the incline plane in the CPF group relative to that recorded in S/oil ($P<0.05$), VE ($P<0.01$) or VE+CPF group. No significant change ($P>0.05$) in the angle of slip in the VE+CPF group relative to that in S/oil or VE group, and between VE group and that of S/oil group. At week 16, there was a significant decrease in the angle of slip off the incline plane in the CPF group relative to that in S/oil ($P<0.05$) or VE ($P<0.01$) group. Although not significant, there was a 6.3% increase in the angle of slip off the incline plane in the VE+CPF group relative to that in CPF group. There was no significant change ($P>0.05$) in the angle of slip off the plane in the S/oil group compared to that in VE or VE+CPF group (Fig. 3).

3.5 Effect of treatments on ladderwalk performance

There was no significant change ($P>0.05$) in the dynamics of the number of missed rungs in the S/oil, VE and VE+CPF groups throughout the study period. There was a significant decrease ($P<0.01$) in the number of missed rungs in the CPF group at day 0 compared to that in week 8 or 16 but no significant change at week 8 compared to that of week 16.

There was no significant change ($P>0.05$) in the number of missed rungs in between the groups at day 0. At week 8, there was a significant decrease ($P<0.01$) in the number of missed rungs in the CPF group compared to that in S/oil or VE group. Although not significant ($P>0.05$), the mean number of missed rungs in the VE+CPF group was 26% higher relative to that recorded in the CPF group. There was a significant decrease ($P<0.01$) in the number of missed rungs in the VE+CPF group compared to that in S/oil or VE group. There was no significant change ($P>0.05$) in the number of missed rungs in the VE group compared to that in S/oil group. At week 16, there was a significant decrease ($P<0.01$) in the number of missed rungs in the CPF group compared to the VE group but no significant change ($P>0.05$) when compared to that recorded in S/oil or VE+CPF group. There was no significant change ($P>0.05$) in the VE+CPF group compared to that in S/oil or VE group.

Similarly, there was no significant change ($P>0.05$) in the number of missed rungs in the VE group compared to that in the S/oil group (Fig. 4).

Fig. 3. Effect of chronic administration of soya oil, vitamin E and/or chlorpyrifos on the dynamics of locomotion efficiency in Wistar rats.

3.6 Effect of treatments on learning acquisition

There was a significant increase ($P<0.01$) in the number of footshocks applied to the CPF group relative to that recorded in the S/oil, VE or VE+CPF group. There was no significant change ($P>0.05$) in the number of footshocks in the VE+CPF group relative to that in S/oil or VE group (Fig. 5).

Fig. 4. Effect of chronic administration of soya oil, vitamin E and/or chlorpyrifos on the dynamics of incline plane performance in Wistar rats.

Fig. 5. Effect of chronic administration of soya oil, vitamin E and/or chlorpyrifos on the learning task in Wistar rats. ^{abc} $P<0.01$ versus S/oil, VE and VE+CPF groups, respectively.

3.7 Effect of treatments on short-term memory

A significant decrease ($P<0.01$) in the duration of stay on platform (latency on platform) was recorded in the CPF group compared to that in the S/oil, VE or VE+CPF group. There was no significant change ($P>0.05$) in the duration of stay on the platform in the VE+CPF group compared to that in the S/oil or VE group (Fig. 6).

3.8 Effect of treatments on brain malonaldehyde concentration

A significant increase ($P<0.01$) in MDA concentration was recorded in the CPF group relative to that in the S/oil, VE or VE+CPF group. There was no significant change ($P>0.05$) in the brain MDA concentration in the VE+CPF group compared to that in S/oil or VE group, nor between VE and S/oil groups (Fig. 7).

3.9 Effect of treatments on brain superoxide dismutase activity

There was a significant decrease ($P<0.01$) in SOD activity in the CPF group relative to the S/oil, VE or VE+CPF group. No significant change ($P>0.05$) was recorded in SOD activity in the VE+CPF group relative to that in S/oil or VE group, nor between VE and that recorded in the S/oil group (Fig. 8).

3.10 Effect of treatments on brain catalase activity

A significant decrease ($P<0.01$) in brain CAT activity was recorded in the CPF group relative that in the S/oil, VE or VE+CPF group. The CAT activity in the VE+CPF group did not

Fig. 6. Effect of chronic administration of soya oil, vitamin E and/or chlorpyrifos on short-term memory in Wistar rats. ^{abc}P<0.01versus S/oil, VE and VE+CPF groups, respectively.

Fig. 7. Effect of chronic administration of soya oil, vitamin E and/or chlorpyrifos on the brain malonaldehyde concentration in Wistar rats. ^{abc}P<0.01versus S/oil, VE and VE+CPF groups, respectively.

differ significantly ($P>0.05$) when compared to that in the S/oil or VE group, and between VE and that recorded in the S/oil group (Fig. 9).

Fig. 8. Effect of chronic administration of soya oil, vitamin E and/or chlorpyrifos on the superoxide dismutase activity in Wistar rats. ^{abc} $P<0.01$ versus S/oil, VE and VE+CPF groups, respectively.

Fig. 9. Effect of chronic administration of soya oil, vitamin E and/or chlorpyrifos on the catalase activity in Wistar rats. ^{abc} $P<0.01$ versus S/oil, VE and VE+CPF groups, respectively

3.11 Effect of treatments on brain acetylcholinesterase activity

There was a significant decrease in brain AChE activity in the CPF group compared to that in the S/oil ($P<0.01$), VE ($P<0.01$) or VE+CPF ($P<0.05$) group. There was no significant change ($P>0.05$) recorded in CAT activity in the VE+CPF relative to that in the S/oil and VE groups, respectively, or between VE and S/oil groups (Fig. 10).

Fig. 10. Effect of chronic administration of soya oil, vitamin E and/or chlorpyrifos on the acetylcholinesterase activity in Wistar rats. ^{ab} $P<0.01$ versus S/oil and VE groups, respectively; ^c $P<0.05$ versus VE group.

4. Discussion

The increase in brain MDA concentration and low SOD and CAT activities in the CPF group is an indication of the ability of this pesticide to elevate lipoperoxidative changes and thereby induce oxidative stress. This was in agreement with the findings from our previous studies (Ambali et al., 2010a; Ambali & Ayo, 2011a, 2011b; Ambali & Aliyu, 2012). The brain due to its biochemical and physiological properties is especially sensitive to free radicals, which destroy its functions and structure (Drewa et al., 1998). The brain is highly vulnerable to oxidative stress because in addition to harboring large amount of oxygen in a relatively small mass, it contains a significant quantity of metals (Fe), and has fewer antioxidant molecules than other organs (Halliwell and Gutteridge, 1999; Naffa-Mazzacoratt et al., 2001). For instance, the CNS is relatively poorly endowed with SOD, CAT, and glutathione peroxidase, and is also relatively lacking in vitamin E (Halliwell & Gutteridge, 1985). CPF is lipophilic and may enhance lipid peroxidation by directly interacting with cellular plasma membrane (Hazarika et al., 2003). The increased MDA concentration which is due to induction of free radical has been shown to alter the composition of membrane lipids, proteins, carbohydrates and DNA. Membrane lipids are vital for the maintenance of cellular integrity and survival (Jain, 1989). Peroxidation of membrane lipids results in the

inactivation of enzymes and cross-linking of membrane lipids and proteins and in cell death (Pfafferott et al., 1982; Jain et al., 1983; Jain, 1984). Furthermore, by-products of lipid peroxidation have been shown to cause profound alterations in the structural organization and functions of the cell membrane including decreased membrane fluidity, increased membrane permeability, inactivation of membrane-bound enzymes and loss of essential fatty acids (Van Ginkel & Sevanian, 1994). This lipoperoxidative changes may cause alterations in the structural and functional components of the brain neuronal cells.

The decrease in the SOD and CAT activities in the CPF group has been reported in previous studies (Tuzmen et al., 2007, 2008; Aly et al., 2010; Ambali & Ayo, 2011a) and may reflect the level of oxidative damage caused by the pesticide. SOD is involved in dismutation of the $O_2^{\bullet-}$ to H_2O_2 and oxygen. The significant reduction recorded in the CPF group may be due to either reduction in its synthesis or elevated degradation or inactivation of the enzyme. CAT, on the other hand is known to neutralize H_2O_2 and covert it to H_2O and O_2 . The significant decline in the CAT activity observed in group exposed to CPF only may be due to the reduced conversion of $O_2^{\bullet-}$ to H_2O_2 by SOD thereby resulting in the accumulation of $O_2^{\bullet-}$. This accumulated $O_2^{\bullet-}$ inhibits the activity of CAT (Kono & Fridovich, 1982). 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 & Chaudhuri, 2002). Furthermore, $O_2^{\bullet-}$ converts ferroxy state of CAT to ferryl state, which is an inactive form of the enzyme (Freeman & Crapo, 1982), thereby exacerbating the free radical-induced damage to the body tissue.

Pretreatment with vitamin E was shown by the present study to reduce the brain MDA concentration and increase the activities of the antioxidant enzymes, SOD and CAT reflecting its antioxidant properties. α -tocopherol prevents the peroxidation of membrane phospholipids and prevent cell membrane damage through its antioxidant action. The lipophilic character of tocopherol makes it easier to locate the interior of the cell membrane bilayer to exert its antioxidant action. Tocopherol-OH transfers a hydrogen atom with a single electron to a free radical, thus removing the radical before it can interact with the cell membrane (Krishnamoorthy et al., 2007). The decreased lipoperoxidation of the membrane due to free radical scavenging effect of vitamin E may have been responsible for the restoration of SOD and CAT activities, since the vitamin may have prevent their full participation in free radical neutralization, hence preserving their activities.

The result also revealed that chronic CPF exposure caused reduction in the brain AChE activity similar to what has been reported in previous studies (Ambali et al., 2010a; Ambali & Ayo, 2011a, 2011b; Ambali & Aliyu, 2012). The ability of CPF to phosphorylate AChE results in impairment of its activity, hence the cholinergic crisis. Apart from this, the induction of lipoperoxidation may have partly contributed to the impaired AChE activity recorded in the CPF group. Oxidative stress affects 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 (Souza et al., 2010). Besides, $OH^{\bullet}$ has been shown to cause significant reduction in AChE activity in the rat brain (Tsakiris et al., 2000). Vitamin E was shown in the present study to restore the activity of AChE probably due to its antioxidant activity. Vitamin E has been shown in previous studies to restore AChE activity impaired by CPF (Yavuz et al., 2004; Ambali & Aliyu, 2012). The lacrimation and intermittent tremors observed in the CPF group is part of the cholinergic syndrome typical of 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. The ability of vitamin E to remedy the CPF-induced cholinergic signs may be attributed its AChE restoration activity. Furthermore, vitamin E has been shown to increase the activity of paraoxonase 1 (Jarvik et al., 2002), an enzyme that increases the detoxification of OP compounds (Shih et al., 1998).

Beam walking across bridges of different cross-sections provides a well-established method of monitoring motor coordination and balance in rodents. The progressive increase in the width at which rats in the CPF group slipped off the beam which indicates impairment of motor coordination has been reported in previous studies (Ambali et al., 2010a; Ambali & Aliyu, 2012). Abou-Donia et al. (2002) observed similar results following repeated exposure of rats to sarin. Beam-walking performance is an integrated form of behavior requiring pertinent level of consciousness, memory, sensorimotor and cortical functions mediated by the cortical area (Abou-Donia et al., 2001). Cortical injury may therefore have been responsible for the deficit in beam-walk performance in the CPF group (Abou-Donia et al., 2001) partly due to oxidative damage. Indeed, CPF and CPF-oxon have been shown to induce apoptosis in rat cortical neuron independent of AChE inhibition (Caughlan et al., 2004). Pretreatment with vitamin E mitigated but did not completely abolish the motor coordination deficits induced by chronic CPF exposure. This is because there was a significant increase in the width at which the VE+CPF group slipped off the beam at week 16 compared to day 0. This shows that oxidative stress may not be the only mechanism involved in motor coordination deficits induced by chronic CPF exposure.

The present study has also shown a significant reduction in forepaw grip time, reflecting deficit in forepaw motor strength following chronic CPF exposure in rats. The result agreed with the finding obtained in an earlier study which showed reduction in hind limb grip strength following repeated CPF administration in rats (Terry et al., 2003). The impairment of motor strength by CPF may have also been due to the decrease in anterograde axonal transport (Terry et al., 2007) or reduced neuronal viability associated with impaired microtubule synthesis and/or function (Prendergast et al., 2007). It has also been postulated that disruption of kinesin-dependent intracellular transport may account for some of the long-term effects of OPs on the peripheral and central nervous system (Gearharta et al., 2007). Reduced hand strength (Miranda et al., 2004) and loss of muscle strength (Steenland et al., 2000) have been observed in humans following prolonged exposure to OPs. Relationship has also been established between higher OP exposure and the development of chronic fatigue syndrome (Tahmaz et al., 2003). Furthermore, the role of muscle (Ambali and Ayo, 2011b) and brain oxidative damage induced by CPF which causes impairment of neuronal viability (Ambali & Ayo, 2011a) hence reduction of motor strength cannot be over emphasized. Although there was a significant deficit in motor strength in the VE+CPF group at weeks 16 and 8 when respectively compared to day 0, the fact that there was no significant change especially at week 16 compared to S/oil and VE groups reflect improvement in motor strength in this group. This may be partly due to reduced brain and perhaps muscle oxidative damage complemented by improvement in AChE activity which improves neuronal transmission.

Chronic CPF exposure has been shown in the present study to interfere with neuromuscular coordination as shown by the decline in the incline plane performance at weeks 8 and 16. The inclined plane test has been used to evaluate integrated muscle function and strength in rodents by evaluating their ability to maintain body position on a board as its angle of inclination is increased. We have earlier demonstrated the ability of acute CPF exposure to impair short-term neuromuscular coordination (Ambali et al., 2010a; Ambali & Aliyu, 2012).

Abou-Donia et al. (2002) similarly showed the ability of the OP warfare agent, sarin to impair incline plane performance in rats. The impairment of neuromuscular coordination may be due to increase in brain oxidative changes induced by CPF, which alters the morphological and functional capacity of the brain region involved in neuromuscular coordination. Oxidative damage to the brain following CPF exposure has been reported in previous studies (Verma, 2001; Ambali et al., 2010a; Ambali & Ayo, 2011a, 2011b; Ambali & Aliyu, 2012). Furthermore, the reduction of AChE activity may have been partly involved in the impaired neuromuscular coordination recorded in the CPF group, since alterations in ACh metabolism may alter neuronal activity.

Although the incline plane performance in the group pretreated with vitamin E at week 16 was significantly lower than that obtained at day 0, the study generally showed that performance in weeks 16 and 8 in the VE+CPF group was not significantly different from that of S/oil or VE group. This shows that the vitamin mitigated the CPF-evoked deficit in neuromuscular coordination. The fact that vitamin E did not completely abolish the CPF-induced impaired incline plane performance shows that oxidative stress and restoration of AChE activity may not be the only factor responsible for the sensorimotor deficit.

The lower ladder score characterized by lower number of missed rungs observed in rats chronically exposed to CPF indicates that the legs of the rats were frequently being held stationary above the rungs for a relatively longer period. This observation demonstrated difficulty in the ability of CPF group to move fast through the obstacles, and hence a deficit in locomotor activity. The deficit in locomotor efficiency observed in the CPF group was dependent on the duration of exposure, with much more impairment recorded at week 16 compared to week 8. The results agreed with the previous findings that slowness of movement is one of the extrapyramidal symptoms (Parkinsonism) observed in humans exposed to non-specific agricultural pesticides, which increased with the duration of exposure (Ritz & Yu, 2000; Alavanja et al., 2004). Thus, the locomotion deficit in the CPF group observed in the present study is part of the sensorimotor deficits occurring in animals chronically exposed to CPF. This impaired mobility may be due to oxidative stress as oxidative damage to the muscle induced by CPF (Ambali & Ayo, 2011b) may have probably caused necrosis thereby impairing locomotion efficiency. Carr et al. (2001) attributed reduced mobility observed in OP poisoning partly to damage in the peripheral musculature, probably due to necrosis of skeletal muscle fibre. Muscle necrosis has been observed following exposure to the OP insecticide, isofenphos and the insecticide metabolite, paraoxon (Dettbarn, 1984; Calore et al., 1999). Similarly, the impaired mobility may be due to inhibition of AChE activity and the subsequent cholinergic paralysis induced by CPF. The severity of the muscle necrosis may be dependent on the level and duration of AChE inhibition (Carr et al., 2001). The amelioration of the locomotor deficits manifested in the improvement of ladder walk and characterized by increase in the number of missed rungs in rats pretreated with vitamin E demonstrated the important role played by oxidative stress and AChE inhibition in the locomotor deficit induced by CPF.

The significant increase in the number of footshocks received by the CPF group relative to the other groups indicates learning impairment. Similarly, the significant reduction in the duration the animal in the CPF group stayed on the platform indicates deficit in memory. This shows that CPF exposure even at low dose is capable of cognitive impairment. CPF-induced cognitive impairment have been reported in several studies in rats (Bushnell et al. 1991; 1994; Prendergast et al., 1997, 1998, 2007; Stone et al., 2000, Moser et al., 2005; Ambali

et al., 2010a; Ambali & Aliyu, 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 the CPF group may be due to alteration in ACh metabolism due to reduction of AChE activity. Since ACh has been demonstrated to be involved in cognition, agents such as OPs which alter ACh metabolism may interfere with this role. 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 & Chambers, 1991).

Furthermore, CPF has been shown to induce cytotoxicity directly on the hippocampal cells via the induction of apoptosis, irrespective of its effect on AChE (Terry et al., 2003). Induction of apoptosis has been described as the toxic end-point of CPF neurotoxicity in the brain as it induces structural changes in the brain that may cause functional deficits, including those involved in memory and learning (Caughlan et al., 2004). Apoptosis probably resulting from oxidative damage to cellular macromolecules may have been responsible for the massive degenerative changes in the brain neurons and glial cells of rats chronically exposed to CPF that we reported in an earlier study (Ambali & Ayo, 2011a). CPF-induced oxidative stress may be central to apoptosis, since free radicals have been implicated in apoptotic death of cells (Corcoran et al., 1994; McConkey et al., 1994). Degenerative changes in the neurons leads to functional deficits as it relates to neurotransmission and other brain activities.

Vitamin E has been shown in the present study to improve learning and short-term memory impaired by chronic CPF exposure. We have earlier demonstrated the ability of either vitamin C or E to mitigate short-term cognitive changes induced by acute CPF exposure in rats (Ambali et al., 2010a; Ambali and Aliyu, 2012). The improved learning and short-term memory recorded following pretreatment with vitamin E may be due to its antioxidant and AChE restoration properties. Apart from its antioxidant function, vitamin E influences the cellular response to oxidative stress through modulation of signal-transduction pathways (Azzi et al., 1992), which may have further enhanced the neuronal function. Similarly, neuroprotective effect of vitamin E has been established in several studies (Frantseva et al., 2000a, 2000b; Pace et al., 2003; El-Hossary et al., 2009) and may have contributed in mitigating the behavioural changes induced by CPF in the present study.

5. Conclusion

The present study has shown that the impaired sensorimotor and cognitive changes induced by chronic CPF exposure mitigated by pretreatment with vitamin E are partly due to its antioxidant, neuroprotective and AChE restoration properties.

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It is our hope that this book will be of interest and use not only to scientists, but also to the food-producing industry, governments, politicians and consumers as well. If we are able to stimulate this interest, albeit in a small way, we have achieved our goal.

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