Sensorimotor and Cognitive Changes Induced by Subchronic Co-Administration of Chlorpyrifos and Lead in Wistar Rats: Mitigating Effect of Vitamin C

Suleiman F. Ambali, Abubakar Woziri, Mufta’u Shittu, Richard Edeh, Chidebere Uchendu, Mohammed M. Suleiman and Lukuman S. Yaqub

1Department of Veterinary Physiology and Pharmacology, University of Ilorin, Ilorin, Nigeria
2Department of Veterinary Pharmacology, Ahmadu Bello University Zaria, Nigeria
3Department of Veterinary Physiology, Ahmadu Bello University Zaria, Nigeria

Abstract: The environment has been invaded by many contaminants, most of which are deliberately released as a result of man’s activity. The effect of multiple contaminants poses new environmental and health challenges to man and animals. Pesticides including organophosphate (OP) and heavy metals including lead are released into the environment as a result of man’s activity. Lead (Pb) and OP are nerve poisons that have been shown to induce neurobehavioral toxicity in man and animals. Oxidative stress is a common mechanism implicated in OP and lead poisoning. The present study was aimed at evaluating the ameliorative effect of antioxidant vitamin C on sensorimotor and cognitive changes induced by co-administration of chlorpyrifos (CPF), an OP insecticide and lead in Wistar rats. Forty adult male Wistar rats divided into 4 groups of 10 animals each were used for the study. Groups I, II, III were given soya oil (2 mg/kg b.wt.), vitamin C (100 mg/kg b.wt) and combination of CPF (4.25 mg/kg~1/20 LD₅₀) and Pb (225 mg/kg~1/20 LD₅₀), respectively. Group IV was pretreated with vitamin C (100 mg/kg b.wt) and then co-administered with CPF (4.25 mg/kg b.wt) and Pb (225 mg/kg b.wt), 30 min later. The regimens were administered by gavage once daily for a period of 9 weeks. The animals were evaluated at various intervals during this period for neurobehavioral signs measuring neuromuscular and motor coordination, motor strength, learning and short-term memory. The animals were sacrificed and the brains evaluated for malonaldehyde (MDA) concentration. The result showed that impairments of neuromuscular and motor coordination, motor strength, learning and memory and an increase in brain MDA concentration induced by co-administration of CPF and Pb were mitigated by vitamin C. The study concludes that vitamin C mitigation of sensorimotor and cognitive deficits induced by co-administration of CPF and Pb was partly due to its anti-lipoperoxidative property.

Key words: Chlorpyrifos · Lead · Co-Administration · Sensorimotor · Cognition · Brain Lipoperoxidation · Amelioration Vitamin C

INTRODUCTION

The increasing technological advancement has been accompanied by the release of multiple chemicals in the environment. Therefore, man and animals are exposed to a “soup” of chemical contaminants in the environment, which directly or indirectly affect their health and well being. The “invasion” of the environment with these multiple chemicals has come with it new environmental and health challenges. Current understanding of the toxicity of many environmental toxicants/pollutants is based primarily on toxicity studies performed on laboratory animals exposed to a single toxic agent [1,2]. However, the reality of multiple chemical contaminants interacting with each other and the environment has brought to the fore the need to investigate the effect of co-exposure to these chemicals on human and animal health.

Pesticides including organophosphate (OP) insecticides are one of the chemicals that are deliberately released into the environment to combat the menace of pests but whose action is not limited to the target organisms. Indeed, OP insecticides constitute about 50% global insecticide use [3], as they are widely used in agriculture, horticulture and also in medicine and industry [4].

Corresponding Author: Dr. S.F. Ambali, Department of Veterinary Physiology and Pharmacology, University of Ilorin, Ilorin, Nigeria. Tel: +234803705411.
Chlorpyrifos (CPF), a chlorinated OP compound is one of the most widely used of the group, despite restriction placed on some of its domestic uses by United States Environmental Agency in 2000 [5]. Due to their wide availability, poisoning by CPF is common [6] and is one the most abundant environmental insecticide contaminants [7,8], as residual amounts have been detected in the soil, water bodies, vegetables, grains and other food products [4]. Lead, is also one of the most pervasive environmental contaminants due to its increasing industrial and even domestic uses. In both developed and developing countries, lead is used by industries in paints, vehicles as an antiknock agent, toys, bullets, plumbing materials, kitchen utensils, among others [9]. Exposure to lead has been known to adversely affect human and animal health in urbanized communities [10]. As nerve poisons, CPF and Pb have been shown individually [11-18] and collectively [19] to cause neurobehavioral changes in rats.

Like many other OP insecticides, the main mechanism of systemic CPF intoxication is related to the acetylcholinesterase (AChE) inhibition. However, toxicity occurs at doses that did not inhibit AChE giving clue as to other possible molecular mechanisms [20-24]. The induction of oxidative stress is one of the molecular mechanisms implicated in OP toxicity [16-18, 25-29]. Pb has multiple mechanisms of action including those related to the induction of oxidative stress [30-32]. The mechanisms of lead-related pathologies, many of which are a direct result of the oxidant effect of Pb on tissues and cellular components, may be mitigated by improving the cellular availability of antioxidants [33].

Since oxidative stress is central to the molecular mechanisms of these two chemicals, we have earlier exploited this in mitigating the hemo- [27] and biochemical [28] toxicity evoked by the co-administration of these two agents. The present study was therefore aimed at evaluating the mitigating effect of antioxidant vitamin C on sensorimotor and cognitive changes evoked by subchronic co-administration of CPF and Pb in Wistar rats.

**MATERIALS AND METHODS**

**Experimental Animals:** Forty male Wistar rats weighing 132-146g were obtained from the Laboratory Animal House of the Department of Veterinary Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The rats were housed in metal cages under standard environmental conditions in the Toxicology Laboratory of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The rats were fed standard rat chow and water was provided *ad libitum*. The procedures used were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals [34].

**Chemicals:** Commercial grade chlorpyrifos (Termicot®; 20% EC, Saberos Organics Gujarat Ltd, India) was prepared by reconstituting in corn oil (C/oil). Analytical grade lead acetate (Kiran Light Laboratories, Mumbai, India) used for the study was made into a 20% stock solution using distilled water. Commercial grade vitamin C tablets (Emzor Pharmaceutical Ltd, Nigeria, BN: 618N) were prepared in distilled water to make 10% stock solution just prior to daily administration.

**Animal Treatments:** Forty weaned male Wistar rats were divided at random into four groups of 10 animals per group. Group I (C/oil) was administered corn oil (2 ml/kg b.wt.), while group II (VC) was given vitamin C (100 mg/kg b.wt.). Group III (CPF+Pb) was co-administered CPF (4.25 mg/kg~1/20 LD₅₀ [35]) and lead acetate (225 mg/kg~1/20 LD₅₀ [15]), respectively. Group IV was pretreated vitamin C (100 mg/kg b.wt.) and then co-administered CPF (4.25 mg/kg b.wt.) and Pb (225 mg/kg b.wt.), 30 min later. These regimens were administered orally by gavage once daily for a period of 9 weeks. During this period, the rats were examined at various intervals for neurobehavioural signs measuring neuromuscular and motor coordination, motor strength, learning and short-term memory. The neurobehavioral parameters were conducted by two trained raters blinded to the treatments to avoid bias. At the termination of the treatments, the animals were killed via jugular venesection after light chloroform anesthesia. The brain was quickly dissected out and evaluated for malonaldehyde (MDA) concentration.

**Evaluation of the Effect of Treatments on Neuromuscular Coordination:** The effect of the treatments on neuromuscular coordination was assessed using the inclined plane apparatus as described by Ambali *et al.* [14]. Briefly, each rat was placed on an apparatus made with an angled rough wooden plank, with a thick foam pad at its bottom end to serve as cushion. The plank was set at an inclination of 35° and was increased 5° stepwise until the animal could no longer stay horizontally on the platform for 30 sec without sliding down the plane.
Angles were increased and marked prior to the start of the experiment and were obtained by placing the plank on two wooden bars with several notches and a metal rod inserted into the notched holes on the vertical wooden bars. The test was performed with the rat facing left and then right. The highest angles at which the rats could stay for 30 sec. were recorded accordingly. Two trials were performed for each testing session and the average angle was recorded for each animal. The test was conducted on day 0 and weeks 3, 6 and 9.

Evaluation of the Effect of Treatments on Motor Coordination: The effect of treatments on motor coordination was performed using the beam walk apparatus as described by Ambali et al. [14]. Briefly, each rat 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, the width of the beam at which they stepped down was recorded by one rater on each side and this was repeated twice during each trial session. This was conducted on day 0 and weeks 3, 6 and 9.

Evaluation of the Effect of Treatments on Motor Strength: The forepaw grip time was used to evaluate the motor strength of the rats using the grip test apparatus as described by Abou-Donia et al. [36]. This was conducted by having rats hung down from a 5 mm diameter wood 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 and weeks 3, 6 and 9.

Evaluation of the Effect of Treatments on Cognition: The effects of the treatments on learning and short-term memory were evaluated at 48 and 24 hrs, respectively, to the termination of the experiment using the step-down inhibitory avoidance learning device as described by Zhu et al. [37].

Determination of Brain Malonaldehyde Concentration: The evaluation of brain MDA concentration as an indication of lipid peroxidation was performed using the double heating method of Draper and Hadley [38] as modified by Freitas et al. [39]. The principle of this method is based on spectrophotometric measurement of the color developed during the reaction of thiobarbituric acid (TBA) with MDA. The concentration of MDA in the brain tissues was calculated by the absorbance coefficient of MDA-TBA complex (1.56 × 10^5 cm^−1 M^−1) and expressed as nmol/mg of tissue protein. Tissue protein concentration was evaluated using the method of Lowry et al. [40].

Statistical Analysis: Data obtained from neurobehavioral parameters measured repeatedly expressed as mean ± SEM were subjected to repeated measure analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. Data obtained from neurobehavioral parameters measured once in the study and those obtained from biochemical parameters were analyzed using one-way analysis of variance followed by Tukey’s test using Graphpad prism version 4.0 for Window. Values of P < 0.05 were considered significant.

RESULTS

Effect of Treatments on Neuromuscular Coordination: The effect of treatments on the inclined plane dynamics is shown in Figure 1. There was a significant decrease in the angle of slip in the CPF+Pb group at week 6 (P<0.05) and week 8 (P<0.01) when respectively compared to that recorded in day 0. There was no significant (P>0.05) change in the angle of slip in the C/oil, VC or VC+CPF+Pb group when the weeks of evaluation in each of the groups were compared between each other.

There was no significant (P>0.05) change in the angle of slip in between the groups at day 0. However, at weeks 3, 6 and 9, the angles of slip in the CPF+Pb group were significantly (P<0.01) lower compared to that of C/oil, VC or VC+CPF+Pb group. Similarly, the angles of slip in the VC+CPF+Pb group at weeks 3, 6 and 9 were significantly (P<0.01) lower when compared to C/oil or VC group.

Effect of Treatments on Motor Coordination: The effect of treatments on the beam walk dynamics is shown in Figure 2. There were no significant (P>0.05) changes in the width of slip off the beam in the C/oil, VC or VC+CPF+Pb group when the days of evaluation were compared between each other. The width of slip off the beam was significantly (P<0.01) lower in the CPF+Pb group at weeks 6 and 9 when respectively compared to that recorded at day 0. The angle of slip in the CPF+Pb group was significantly (P<0.01) lower at week 9 compared to that recorded at week 3.
Effect of Treatments on Motor Strength: The effect of the treatments on dynamics of motor strength is shown in Figure 3. There was no significant (P>0.05) change in the grip time recorded in the C/oil and VC groups in between the periods of evaluation. There was a significant (P<0.05) increase in the grip time of VC group at week 9 compared to that of day 0. There was a significant decrease in the grip time in the CPF+Pb group at week 9 when compared to that recorded on day 0 (P<0.05) and week 3 (P<0.01). There was no significant (P>0.05) change in the grip time in between the groups at week 9 except the significant (P<0.01) increase recorded in the VC group relative to that of CPF+Pb group.
Fig. 3: Effect of corn oil (C/oil), vitamin C (VC) and/or chlorpyrifos+lead (CPF+Pb) on grip time dynamics in Wistar rats.

Fig. 4: Effect of corn oil (C/oil), vitamin C (VC) and/or chlorpyrifos+lead (CPF+Pb) on learning acquisition in Wistar rats. *P<0.01 vs C/oil and VC groups, respectively

Effect of Treatments on Learning Acquisition:
There was a significant increase (P<0.05) in the number of footshocks in the CPF + Pb group compared to that of C/oil and VC groups, respectively. Although not significant (P>0.05), the number of footshocks applied to CPF+Pb group was 35% higher than that VC+CPF+Pb group. There was no significant (P>0.05) change in the number of footshocks applied to VC+CPF+Pb group relative to that of C/oil or VC group (Figure 4).

Effect of Treatments on Short-Term Memory:
The effect of the treatments on short-term memory is shown in Figure 5. The latency on platform was significantly (P<0.05) lower in the CPF + Pb group compared to that of the C/oil or VC group. Although not significant (P>0.05), the latency on platform in the VC+CPF+Pb group was 28% higher than that recorded in the CPF+Pb group. There was no significant (P>0.05) change in the latency on platform in the VC+CPF+Pb group relative to that of C/oil or VC group.

Effect of Treatments on Brain Malonaldehyde Concentration:
The effect of treatments on whole brain MDA concentration is shown in Figure 6. There was a significant (P<0.01) increase in the brain MDA concentration in the CPF + Pb group when compared to that of C/oil, VC or VC + CPF + Pb group. There was no significant (P>0.05) change in the brain MDA concentration in the VC+CPF+Pb group relative to that of C/oil or VC group.
DISCUSSION

The inclined plane is used to access neuromuscular coordination. The result of this present study revealed a deficit in incline plane performance, hence neuromuscular coordination following co-administration of CPF and Pb. Previous studies have shown deficit in neuromuscular coordination measured by incline plane performance following CPF [14, 18] and Pb [15] exposure. This may have been due to oxidative damage by these chemicals to the cerebral cortex. The fact that pretreatment with vitamin C improved the incline plane performance hence neuromuscular coordination further demonstrate that oxidative damage may have partly contributed to the deficit incline plane performance following co-administration of CPF and Pb. Earlier studies have shown the mitigating effect of vitamin C on deficit in incline plane performance evoked by CPF [14] and Pb [15] exposure.

The co-administration of CPF and Pb has also been shown in the present study to impair motor coordination as shown by deficit in beam walk performance. Studies have shown that CPF [14,18] and Pb [15] impaired motor coordination in rats. The deficit in motor coordination may
have been due to cortical injury [36] evoked by co-administration of CPF and Pb. In addition, the deficit may have arisen from low norepinephrine production since the hormone produced by locus coeruleus has been shown to facilitate recovery from locomotor deficits [41]. Therefore, oxidative injury to cerebral cortex and locus coeruleus induced by CPF [14,16] and lead [10,15,42] may have been partly responsible for the deficits in motor coordination in this group.

The present study revealed a lower motor strength in rats co-administered with CPF and Pb as shown by reduced grip time. This may have been due to neuronal damage since CPF has been shown to cause oxidative damage to the neurons [16,44]. Similarly, necrosis of the muscle induced by prolonged AChE inhibition hence persistent ACh activity [45] and perhaps oxidative damage to the muscle [17] may have contributed to the reduced motor strength in rats co-administered CPF and Pb. The improvement in motor strength following pretreatment with vitamin C suggests that the neuronal lesions may have been mitigated by the antioxidant vitamin. This is consistent with the findings of El-Hossary et al. [44] and Ambali and Ayo [16] that showed that vitamin C mitigates the neuronal degeneration evoked by CPF.

The study also revealed a significant deficit in learning acquisition following co-administration of CPF and Pb. This is consistent with the findings from previous studies which observed learning deficits following CPF exposure [12,26,46]. This deficit may have arisen following neuronal injury partly due to induction of oxidative stress, especially in the cerebral cortex. El-Hossary et al. [44] and Ambali and Ayo [16] have shown that neuronal damage in the cerebral cortex induced by CPF was mitigated by vitamin C. Lead-induced deficit in learning ability mediated by glutamergic and dopaminergic systems has been demonstrated in rodents [47]. Similarly, co-administration of CPF and Pb resulted in impairment in short-term memory. Impairment of memory has been reported following CPF [14,19,26,46] and Pb [15,48-51] exposure. The cognitive decline may have been partly due to oxidative damage to the hippocampus since long-term potentiation in the hippocampal formation plays an important role in learning and memory processes [52]. Repeated administration of CPF has been shown to impair water maze task [53], a learning paradigm dependent upon intact hippocampal function [54]. Lead has also been shown to concentrate in the hippocampus [55] and cause damage. The affinity of Pb for many calcium binding sites in the brain, which play important role in neuronal maturation and synaptic plasticity [56] may have contributed to Pb-induced cognitive decline. Similarly, inhibition of AChE activity may have played a role in the cognitive decline since several studies have linked cognitive decline in OP poisoning to alteration in ACh metabolism [57,58]. Pb has equally been shown to inhibit AChE activity [59-61] and may also play a role in the cognitive decline induced by these two agents. The alteration in AChE activity may also be linked to induction of oxidative stress since lipid peroxidation has been shown to impair the activities of membrane-bound enzymes, including AChE [62,63]. Impairment of membrane-bound enzymes during oxidative stress may stem from the direct attack of the enzymes by free radicals or peroxidation of the membrane lipids in which they are embedded [64].

The result of the present study showed a significant increase in the brain MDA concentration in the animals that were co-administered CPF and Pb acetate as compared to the other groups. MDA is a function of oxidative stress in the pathophysiology of the deficit in incline plane performance elicited by co-administration of CPF and Pb. Improvement in motor coordination performance has been observed following vitamin C pretreatment of rats exposed to CPF [14,18] and Pb [15]. This may be due to amelioration by vitamin C of the cortical and locus coeruleus injuries evoked by CPF and Pb. Besides, vitamin C has been shown to play an essential role in the synthesis of norepinephrine [43], which eventually ensures the availability of the hormone necessary for the successful performance of beam walk.

The improvement in motor coordination following pretreatment with vitamin C underscores the role of oxidative stress in the pathophysiology of the deficit in incline plane performance elicited by co-administration of CPF and Pb. Improvement in motor coordination performance has been observed following vitamin C pretreatment of rats exposed to CPF [14,18] and Pb [15]. This may be due to amelioration by vitamin C of the cortical and locus coeruleus injuries evoked by CPF and Pb. Besides, vitamin C has been shown to play an essential role in the synthesis of norepinephrine [43], which eventually ensures the availability of the hormone necessary for the successful performance of beam walk.

The improvement in cognitive performance following pretreatment with vitamin C underlies the significance of oxidative stress in the pathogenesis of CPF-induced cognitive decline. Previous studies have shown that vitamin C pretreatment improved the cognitive decline evoked by CPF [14,18] and Pb [15.] exposure. This may have been due to the antioxidant property of the vitamin. Antioxidants have been shown to inhibit apoptosis [65], a mechanism that have been proposed to be contributing to OP-evoked neurotoxicity [66]. Similarly, vitamin C has been shown to aid in the restoration of AChE activity [14,16,67] partly due to its anti-lipoperoxidative effect. All these may have contributed to the ability of the vitamin to restore the cognitive dysfunction evoked by co-administration of CPF and Pb. The result of the present study showed a significant increase in the brain MDA concentration in the animals that were co-administered CPF and Pb acetate as compared to the other groups. MDA is a function of lipoperoxidative changes in tissues. The increased MDA concentration in this group of animals therefore suggests increased brain lipoperoxidation. Elevation of brain lipid
peroxidation in the present study suggests participation of free-radical induced oxidative cell injury in mediating the sensorimotor and cognitive decline evoked by co-administration of CPF and Pb. Oxidative damage has been associated with the molecular mechanism of both CPF [14,16,26,68] Pb [32,33,69] poisoning. The brain is highly vulnerable to oxidative stress due to its high metabolic rate, high levels of polyunsaturated fatty acids and ions, as well as low levels of antioxidants such as SOD and GSH, among others [70]. CPF and Pb have the potential to easily permeate the blood brain barrier, therefore making the brain susceptible to free radical damage and hence oxidative stress. Vitamin C has been shown by the present study to reduce the MDA concentration significantly due to its antioxidant effect. This is in accord with the findings from previous studies where vitamin C was shown to mitigate the oxidative changes evoked by CPF [14,16,17] and Pb [15,71]. Apart from preventing oxidative damage, other mechanisms unrelated to the antioxidant effect of vitamin C may have aided the mitigation of sensorimotor and cognitive decline provoked by co-administration of CPF and Pb. For instance, vitamin C is known to increase the activity of paraoxonase I [72], an essential enzyme that aid OP detoxification. Vitamin C also decreases intestinal absorption of Pb and by reducing ferric ion to ferrous ion in the duodenum, it increases the availability of iron, which compete with Pb for intestinal absorption [73]. Vitamin C also inhibit cellular uptake of Pb [74] thereby decreasing body Pb level.

In conclusion, the present study has shown that vitamin C ameliorated the sensorimotor and cognitive decline evoked by subchronic co-administration of CPF and Pb partly due to its antioxidant property.

REFERENCES


34. Guide for the care and use of laboratory animals, DHEW Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892.


