

Vitamin C Attenuates Chronic Chlorpyrifos-induced Alteration of Neurobehavioral Parameters in Wistar Rats

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ABSTRACT

Background: Oxidative stress is one of the molecular mechanisms in chlorpyrifos toxicity. The present study was designed to evaluate the attenuating effect of vitamin C on chlorpyrifos-induced alteration of neurobehavioral performance and the role of muscle acetylcholinesterase (AChE), glycogen and lipoperoxidation in the accomplishment of this task. **Materials and Methods:** Male rats were randomly assigned into 4 groups with the following regimens: soya oil (S/oil), vitamin C (VC), chlorpyrifos (CPF) and vitamin C+CPF (VC+CPF). The regimens were administered by gavage once daily for a period of 17 weeks. Neurobehavioral parameters measuring efficiency of locomotion, motor strength, righting reflex and excitability were evaluated at day 0 (pretreatment value), weeks 8 and 16. The rats were sacrificed at week 17 and evaluated for muscle glycogen and malonaldehyde (MDA) concentrations and AChE activity. **Results:** The result showed that deficits in locomotion efficiency, motor strength, righting reflex and excitability score induced by chronic CPF were mitigated but not completely abolished by vitamin C. The reduced muscle AChE activity and concentrations of glycogen and MDA evoked by chronic CPF were ameliorated by vitamin C. **Conclusion:** The study therefore showed that improvement in muscle AChE activity, glycogen concentration and reduced lipoperoxidation by vitamin C may be partly responsible for the mitigation of the chronic CPF-induced sensorimotor performance.

Key words: Attenuation, chlorpyrifos, lipoperoxidation, sensorimotor performance, vitamin C

INTRODUCTION

Pesticides including organophosphates (OP) are one of the most widely available environmental chemical contaminants because of their wide use in agriculture, horticulture, and public health. Their exposure has been shown to affect sensorimotor performance in both the young^[1,2] and adult^[3,4] humans and animals. The mechanism of OP-induced neurotoxicity is commonly

related to inhibition of acetylcholinesterase (AChE) leading to accumulation of acetylcholine (ACh) in the central and peripheral nervous systems. This results in overstimulation of muscarinic, nicotinic, and central cholinergic receptors.^[5] However, studies have shown that neurotoxicity occurs at doses that do not inhibit AChE,^[6] prompting investigations into other possible mechanisms. The induction of oxidative stress is one of the molecular mechanisms that have been implicated in OP-evoked neurotoxicity.^[4,7,8] Many studies in both humans and animals have shown that moderate exposure to pesticides is associated with neurologic symptoms, including those of affect, cognitive, motor, sensory, and autonomic functions.^[9,10] Although the short- and long-term neurotoxic sequelae arising from acute OP exposure has been widely documented in the literature,^[8,11,12] data linking the effect of repeated exposure to OP on long-term neurobehavioral changes have been less forthcoming.^[13,14]

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Chlorpyrifos (CPF, O, O-O-[diethyl-3, 5, 6-trichloro-2-pyridyl] phosphorothioate) is a widely used chlorinated OP insecticide that has had some of its indoor uses banned by US Environmental Protection Agency in 2000 due to its neurotoxic effect, especially in children. Despite this restriction, CPF remains a popular insecticide throughout the world.^[15] Like the other OP insecticides, the inhibition of AChE is principally involved in the neurotoxicity induced by this agent. However, oxidative stress is being increasingly shown to play a role in the neurotoxicity instigated by this agent.^[6,8,16] Oxidative stress, which involves accumulation of reactive oxygen and nitrogen species beyond the body's natural antioxidant capacity to detoxify them has been implicated in the pathogenesis of many neurological and neurodegenerative diseases.^[17-19]

Vitamin C is the most widely available water soluble antioxidant molecule that has shown tremendous promise in attenuating OP-induced hematological, biochemical, and histopathological alterations.^[20-22] In recent years, there has been increasing interest in the beneficial effects of antioxidant vitamin C on behavioral aberrations, especially those associated with neurodegenerative diseases.^[23] Therefore, the present study was designed to evaluate the ameliorative effect of vitamin C in attenuating the deficit in sensorimotor performance induced by chronic exposure to CPF in Wistar rats and the contribution of muscle lipoperoxidation, glycogen concentration, and AChE activity in achieving this objective.

MATERIALS AND METHODS

Experimental animals and housing

Twenty 10-week-old male Wistar rats (104 ± 4.2 g) 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 2 weeks in the laboratory prior to the commencement of the experiment. They were fed on standard rat pellets and water was provided *ad libitum*.

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 1 ml of distilled water to obtain 100 mg/ml suspension, just prior its daily administration.

Animal treatment schedule

The rats were weighed and then assigned at random into

four groups of five rats in each group. Rats in group I (S/oil) served as the control and were given only soya oil (2 ml/kg b.w.) while those in group II (VC) were dosed with vitamin C (100 mg/kg b.w.). Rats in group III (CPF) were 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.^[24] Rats in group IV (VC+CPF) were pretreated with vitamin C (100 mg/kg b.w.), and then dosed with CPF (10.6 mg/kg b.w.), 30 minutes later. The regimens were administered once daily by oral gavage for a period of 17 weeks. The animals were evaluated for neurobehavioral parameters that are related to efficiency of locomotion, motor strength, surface righting reflex and excitability at day 0 (pre-exposure), weeks 8 and 16. 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 and the biceps brachii muscle was evaluated for muscle glycogen and malonaldehyde (MDA) concentrations and AChE activity. The procedures used were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).^[25]

Evaluation of the effect of treatments on efficiency of locomotion

The ladder walk as described by Petrich^[26] was used to evaluate the efficiency of locomotion. Briefly, each rat was encouraged to walk across a black wooden ladder (106 cm \times 17 cm, with 0.8-cm diameter rungs, and with 2.5-cm spaces between them). The number of times each rat missed a rung was counted by one rater on each side. This neurobehavioral parameter was evaluated on day 0, weeks 8 and 16 between 7 hours and 10 hours.

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.*^[27] 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, weeks 8, and 16 between 7 hours and 10 hours.

Effect of treatments on surface righting reflex

The surface righting reflex was assessed by holding a rat at the base of its tail on a leveled flat surface. With a rapid clockwise motion of the hand, the rat was turned from a prone to a supine position.^[28] The time needed to return to the normal position was noted and rated on an ordinal scale of 0, 1, and 2, thus

Grade 0: Animal with a normal reflex that had ability to right its reflex immediately (within 2 seconds)

Grade 1: Animal with a slightly impaired motor coordination reflex that was able to right its reflex within 2-5 seconds.

Grade 2: Animal with markedly impaired surface righting reflex, which was able to right itself in more than 5 seconds.

The surface righting reflex was evaluated on day 0, weeks 8, and 16 of the study between 7 hours and 10 hours.

Assessment of the level of excitability scores

This neurobehavioral parameter was evaluated using the excitability scores as described by Ayo *et al.*^[29] with modifications. Briefly, each rat was held by the tail upside down and held in that position for 30 seconds. Response of each rat was then rated using an ordinal scale of 0-5 as follows:

Grade 0- Rat did not show any form of wriggling at all.

Grade 1- Rat's wriggling was low with feeble forepaw movement.

Grade 2- Rat responded through a stronger wriggling and forepaw movement.

Grade 3- Rat vigorously wriggled and a strong fore- and hind-limbs movement

Grade 4- In addition to observation in grade 3 above, the rat made unsuccessful attempt to climb on its tail.

Grade 5- In addition to observations in grade 3 above, the rat successfully climbed the tip of its tail.

The excitability score was assessed on day 0, weeks 8, and 16 of the study between 8 hours and 10 hours.

Evaluation of biceps brachii muscle glycogen concentration

The biceps brachii muscle glycogen concentration was evaluated using the method described by Good *et al.*^[30] Briefly, 0.5 g of forelimb muscle was dissected from each animal and was extracted with 3 ml of 30% KOH, incubated for 30 minutes at 100°C, and then brought to acid pH by addition of 20% trichloroacetic acid. The precipitated protein was removed by centrifugation for 10 minutes at 3000×g. Glycogen was precipitated by the addition of 2 ml absolute ethanol and weighed. The results were expressed in g of glycogen/100 g of muscle.

Determination of biceps brachii muscle malonaldehyde concentration

The MDA concentration of the biceps brachii muscle was assayed using the double heating method of Draper and Hadley.^[31] A total of 0.3 g of freshly removed biceps brachii muscle was homogenized in 30 ml cold phosphate buffered saline and centrifuged at 3000×g for 10 minutes. The supernatant from each homogenate was divided into two parts, for MDA and protein concentrations, respectively. The protein concentration was determined using the Lowry method. For the determination of MDA concentrations, 0.25 ml of supernatant was mixed with 0.5 ml of 10%

trichloroacetic acid, and then heated in a boiling water bath for 15 minutes. After cooling under running tap water for 5 minutes, the mixture was centrifuged at 1600×g for 10 minutes; 1 ml of the supernatant was then added to 0.5 ml of 6.7 g/L TBA solution in a test tube and placed in a boiling water bath for 15 minutes. The solution was then cooled under running tap water and the absorbance was then measured at 532 nm using a UV spectrophotometer (T80+UV/VIS Spectrometer® PG Instruments Ltd., UK). The MDA concentration was calculated by the absorbance coefficient, MDA-TBA complex $1.56 \times 10^5/\text{cm}$ and expressed in nmol/mg of protein.

Evaluation of the effect of treatments on biceps brachii acetylcholinesterase activity

Muscle acetylcholinesterase activity was evaluated using the method of Ellman *et al.*^[32] with acetylthiocholine iodide as a substrate. Briefly, 1 g of the muscle of the forearm of each animal was homogenized in a 10 ml cold (0–4°C) 20 mM phosphate buffer saline (PBS). It was incubated with 0.01 M 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 minutes. Then, acetylthiocholine iodide (0.075 M in 0.1 M PBS, pH 8.0) was added, and absorbance at 412 nm was measured continuously for 30 minutes using a UV spectrophotometer (T80+ UV/VIS spectrometer®, PG Instruments Ltd., Leicestershire, LE 175BE, UK). AChE activity expressed as IU/min/mg tissue protein was calculated based on the rate of color change per minute.

Statistical analysis

Data were expressed as mean ± standard error of mean. The ladder walk and grip time performances were analyzed using repeated one-way analysis of variance followed by Tukey's test. The non-parametric data measuring surface righting reflex and excitability scores were analysed using Kruskal-Wallis one-way analysis of variance on ranks followed by Dunn's test, The AChE activity and concentrations of MDA and glycogen were analyzed using one-way analysis of variance followed by Tukey's *post hoc* test. values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSIONS

Effect of treatments on bicep brachii muscle malonaldehyde concentration

There was a significant elevation in the MDA concentration in the CPF group when compared to S/oil ($P < 0.01$), VC ($P < 0.01$), or VC+CPF ($P < 0.05$) group. There was a significant elevation in the MDA concentration in the VC+CPF group compared to that in the S/oil ($P < 0.05$) or VC ($P < 0.01$) group. There was no significant change ($P > 0.05$) in the MDA concentration in the VC group compared to the S/oil group [Figure 1].

The increased lipoperoxidative changes in the biceps brachii muscle in the CPF group further demonstrates the role of oxidative stress in CPF-induced toxicity. Our earlier study has shown that chronic CPF exposure caused increased brain lipoperoxidation and reductions in the activities of brain antioxidant enzymes, superoxide dismutase, and catalase.^[33] The increased muscle lipoperoxidative damage in the CPF group interferes with the integrity of the sarcolemma and hence ion transportation within the muscle membrane. Oxygen free radicals through their destructive effects on biological membranes have been shown to cause disturbances in physiological and biochemical processes, such as mitochondrial oxidative phosphorylation, ion and membrane transport and maintenance of electrical potential difference.^[34] Vitamin C has been shown to reduce the level of membrane lipoperoxidation induced by chronic CPF exposure in the present study apparently due to its antioxidant properties.

Effect of treatments on glycogen concentration

The effect of treatments on biceps brachii muscle glycogen concentration is shown in Figure 2. There was a significant decrease ($P < 0.05$) in the muscle glycogen concentration in the CPF group when compared to that in the S/oil or VC group. There was no significant change ($P > 0.05$) in the glycogen concentration in the VC+CPF group compared to that in the VC or S/oil group. Although not significant, the muscle glycogen in the VC+CPF group increased by 22.4% over that recorded in the CPF group. There was no significant change ($P > 0.05$) in the glycogen concentration in the VC group compared to the S/oil group.

The significantly low biceps brachii muscle glycogen concentration in the CPF group may have been due to deficit in glycogen synthesis apparently resulting from CPF-induced lipoperoxidative changes in the muscle. This is because oxidative stress has been shown to impair glycogen synthesis.^[35] Similarly, CPF has been shown to induce hepatic lesion,^[20,24,36] which further interferes with glycogen synthesis. Furthermore, CPF-induced reactive oxygen species manifested as increased MDA concentration in the present study is known to destroy carbohydrates, including glycogen present in the cellular compartments^[37,38] thereby reducing its tissue concentration. Pretreatment with vitamin C improved the muscle glycogen apparently due to decrease in lipoperoxidative changes in the muscle recorded in the present study and reduction in the severity of CPF-induced hepatic damage by the vitamin that had earlier been demonstrated.^[24] Zhu *et al.*^[39] reported that vitamin C attenuates noise-induced hepatic glycogen reduction in rats. The improvement in muscle glycogen in the group pretreated with vitamin C in the present study may have arisen from the radical scavenging effect of the vitamin thereby protecting the glycogen from the destructive effect of radical.

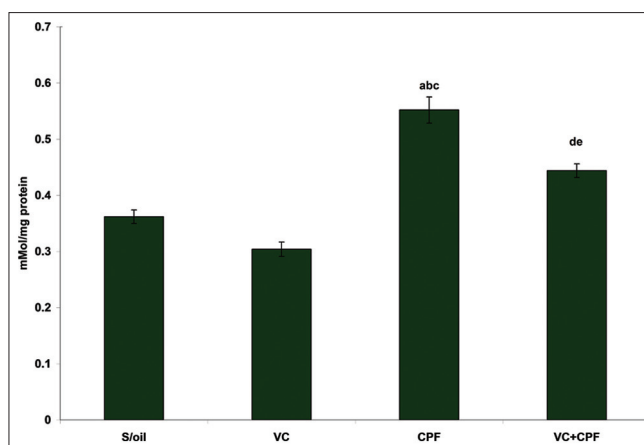


Figure 1: Effect of chronic exposure of Wistar rats to soya oil (S/oil), chlorpyrifos (CPF), and/or vitamin C (VC) on biceps brachii muscle malonaldehyde concentration. ^{ab} $P < 0.01$ vs. S/oil and VC group, respectively, ^c $P < 0.05$ vs. VC+CPF group; ^{de} $P < 0.01$ vs. S/oil and VC, respectively ($n = 5$ animals per group)

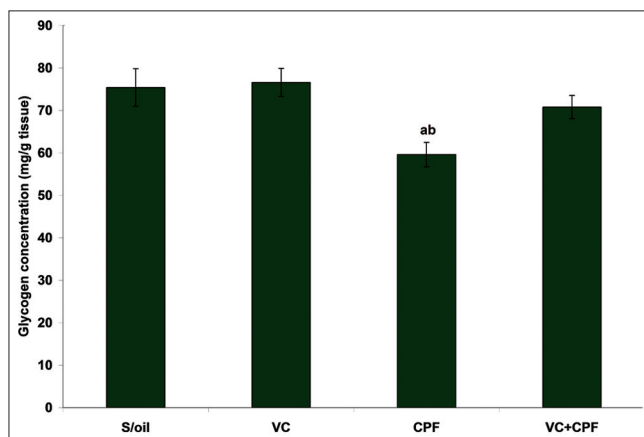


Figure 2: Effect of chronic exposure of Wistar rats to soya oil (S/oil), chlorpyrifos (CPF), and/or vitamin C (VC) on biceps brachii muscle glycogen concentration. ^a $P < 0.05$ vs. S/oil group, ^b $P < 0.05$ vs. VC group ($n = 5$ animals per group)

Effect of treatments on biceps brachii muscle acetylcholinesterase activity

The effect of treatments on biceps brachii muscle AChE activity is presented in Figure 3. There was a significant decrease ($P < 0.01$) in AChE activity in the CPF group compared to that in the S/oil or VC group. The AChE activity in the CPF group was not significantly different ($P > 0.05$) from the VC+CPF group, although there was an 11% increase in AChE activity in the latter group compared to the former. There was no significant change ($P > 0.05$) in the muscle AChE activity in the VC group compared to the S/oil group.

The inhibition of biceps brachii muscle AChE activity by CPF results in ACh accumulation at the cholinergic receptors leading to initial excitation followed by paralysis of muscular activity. This may have contributed to

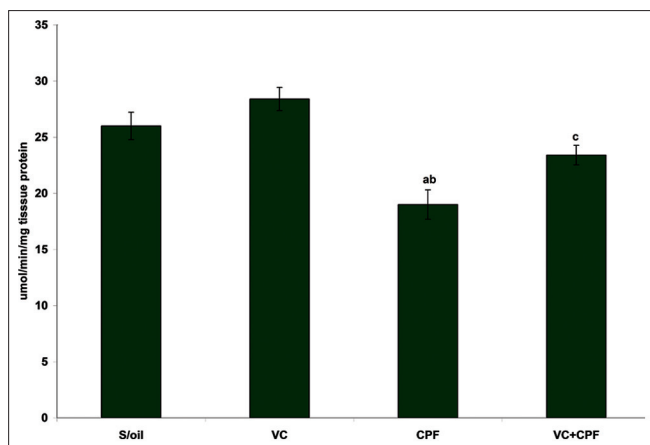


Figure 3: Effect of chronic exposure of Wistar rats to soya oil (S/oil), chlorpyrifos (CPF), and/or vitamin C (VC) on biceps brachii muscle acetylcholinesterase activity. ^{ab} $P < 0.01$ vs. S/oil and VC groups, respectively; ^c $P < 0.05$ vs. VC group ($n = 5$ animals per group)

the alteration in efficiency of locomotion activity and impairment of motor strength recorded in the CPF group. Vitamin C pretreatment resulted in improvement of AChE activity that eventually led to the improvement in neuromuscular activity depressed by chronic CPE. The improved AChE activity in vitamin pretreated group may be due to AChE restoration properties of vitamin C which has been demonstrated in previous studies.^[8,40]

Effect of treatments on efficiency of locomotion

The number of missed rungs in the CPF group increased significantly ($P < 0.01$) at day 0 compared to that at week 8 or 16. There was no significant change ($P > 0.05$) in the number of missed rungs at week 8 compared to week 16 in the CPF group [Figure 4]. There was no significant difference ($P > 0.05$) in the number of missed rungs recorded in the S/oil, VC, and VC+CPF groups at day 0 compared to those of either week 8 or 16. No significant change ($P > 0.05$) in the number of missed rung was also recorded in the VC+CPF group at week 8 compared to 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 the S/oil or VC group but no significant change ($P > 0.05$) compared to VC+CPF group. There was a significant decrease ($P < 0.01$) in the number of missed rungs in the VC+CPF group compared to that in the S/oil or VC group.

The lower ladder walk score characterized by the significantly 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

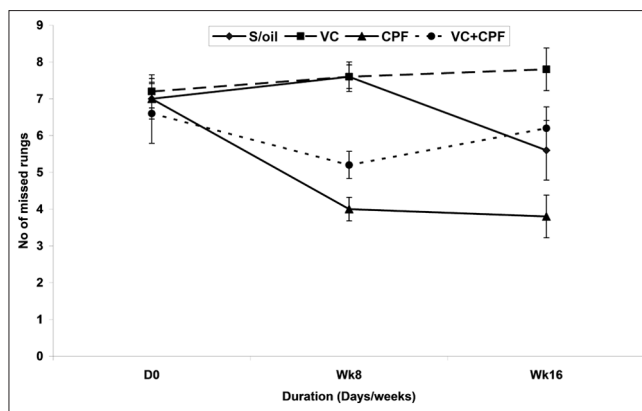


Figure 4: Effects of chronic soya oil (S/oil), vitamin C (VC), and/or chlorpyrifos (CPF) on ladder walk dynamics in male Wistar rats ($n = 5$ animals per group)

demonstrates difficulty in the ability of the rats exposed to CPF only to move fast through the obstacle, and hence a deficit in locomotion activity. The locomotion deficit observed was dependent on the duration of exposure, with worst locomotion performance recorded in week 16. Studies have shown that slowness of movement is one of the extrapyramidal symptoms observed in humans exposed to nonspecific agricultural pesticides, which increased with the duration of exposure.^[13,41] Thus, the locomotion deficit observed in the present study is part of the sensorimotor deficits occurring in animals chronically exposed to CPE. Furthermore, inhibition of AChE activity by CPF may have exacerbated the locomotion deficit through persistent ACh action at the cholinergic receptors, which may eventually result in paralysis. Similarly, the low glycogen reserve of the muscle in this group may have limited the readily available energy essential in the performance of efficient locomotion. Furthermore, the increased lipoperoxidative changes in the muscle may have altered the membrane integrity hence ionic conductance which is pertinent to the conduct of efficient locomotion. The amelioration of the CPF-induced locomotion deficits in group pretreated with vitamin C was demonstrated by a relative increase in the number of missed rungs, especially at week 16. This demonstrates the important role played by oxidative stress in locomotion deficit induced by CPE. Besides, the improvement in AChE activity and increase glycogen availability in the group pretreated with vitamin C may have also complemented the enhancement of locomotion performance.

Effect of treatments on forepaw grip time

The effect of treatments on motor strength as measured by forepaw grip time is shown in Figure 5. There was no significant change in the grip time of rats in the S/oil group when value recorded at day 0 was compared with those obtained at either week 8 or 16. The grip time in the VC group at day 0 was not significantly different ($P > 0.05$) from those recorded at week 8 and week 16. There was a significant increase ($P < 0.01$) in the grip time of rats in

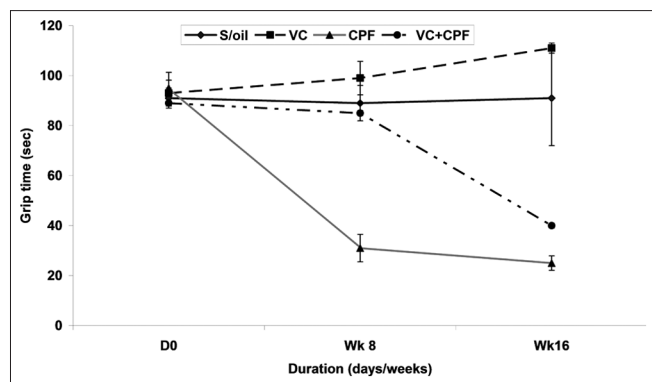


Figure 5: Effect of chronic exposure of Wistar rats to soya oil (S/oil), chlorpyrifos (CPF), and/or vitamin C (VC) on grip time dynamics ($n=5$ animals per group)

the CPF group at day 0 when compared to those obtained at either week 8 or 16. However, there was no significant change ($P>0.05$) in the grip time of CPF group at week 8 compared to week 16. There was no significant difference ($P>0.05$) in the grip time of rats in the VC+CPF group at day 0 when compared to values recorded at week 8 but a significant decrease ($P<0.01$) was recorded in week 16 when compared to that at day 0 or week 8.

At day 0, there was no significant change ($P>0.05$) in the grip time in between the groups. At week 8, there was a significant ($P<0.01$) decrease in the grip time in the CPF group compared to S/oil, VC or VC+CPF group. At week 16, there was a significant ($P<0.05$) decrease in the grip time in the CPF group compared to that in the S/oil or VC group but not VC+CPF group. Similarly, there was a significant decrease in the grip time when the value recorded in the VC+CPF group was compared to that in the S/oil ($P<0.05$) or VC ($P<0.01$) group.

The significant reduction in forepaw grip time, reflecting deficit in forepaw motor strength following chronic CPF exposure agreed with the finding obtained in an earlier study which showed reduction in hind limb grip strength following repeated CPF administration in rats.^[42] Similarly, reduced hand strength,^[43] altered peripheral nerve function,^[44] and loss of muscle strength^[45] have been observed in humans following prolonged exposure to OPs. The reduced grip time following CPF exposure recorded in the present study is an indication of impaired motor strength. This may be related to low glycogen reserve in the muscle of the animals in this group, which eventually affects the energy output required in the performance of this task. Similarly, the increased muscle lipoperoxidative changes may have contributed to the decrease in motor strength in the CPF group due to free radical-induced damage to the muscle. Similarly lipoperoxidation has been shown to affect membrane-bound enzymes including ATPases causing cellular dysfunction, due to alteration of cationic transport across the membranes and disturbance in uptake, as well

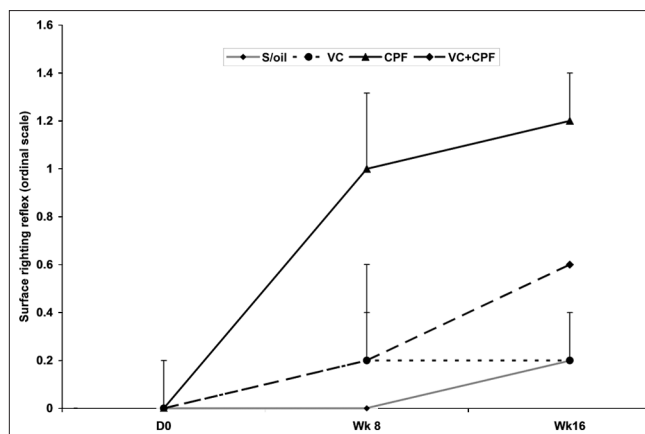


Figure 6: Effect of chronic exposure of Wistar rats to soya oil (S/oil), chlorpyrifos (CPF), and/or vitamin C (VC) on surface righting reflex dynamics ($n=5$ animals per group)

as release of certain neurotransmitters.^[46] In addition, the interference with muscle ACh metabolism resulting from AChE inhibition may have altered neuronal transmission thereby contributing the reduced motor strength. Similarly, the ability of CPF to decrease anterograde axonal transport^[47] may also contribute to the reduced motor strength in the CPF group. The reduced motor strength may be related to the development of chronic fatigue syndrome that has been associated with OP poisoning.^[48]

Pretreatment with vitamin C did not cause a significant change in the CPF-induced impairment of motor strength between day 0 and week 8. However, there was a significant decrease in motor strength when the value obtained in week 16 was compared to that recorded at day 0 or week 8. This showed that the protective effect of vitamin C on motor strength waned with increasing duration of exposure to CPF. However, the fact that the grip time in weeks 8 and 16 in the group pretreated with vitamin C was not significantly different from that in groups exposed to either soya oil or vitamin C only further reinforced some level of protection offered by the vitamin on CPF-evoked deficit in motor strength. This underscores the role of muscle lipoperoxidation, glycogen depletion, and AChE inhibition in the CPF-induced motor deficit.

Effect of treatments on surface righting reflex

The effect of various treatment groups on the dynamics of the reflex righting time is shown in Figure 6. The dynamics showed a progressive increase in the reflex righting time in the CPF and VC+CPF groups, although the magnitude of the increase was comparatively higher in the former. There was no significant change ($P>0.05$) in the reflex righting time when values recorded in day 0 were compared to those of week 8 or 16 in the S/oil, VC, or VC+CPF group. Similarly, there was no significant change ($P>0.05$) in the reflex righting time at day 0 compared to week 8 or at week 8 compared to week 16 in the CPF group. However, there

was a significant difference ($P < 0.05$) in the reflex righting time when values recorded at day 0 was compared to those of week 16 in the CPF group.

There was no significant difference ($P > 0.05$) in reflex righting time in between the groups at day 0. There was a significant delay ($P < 0.05$) in righting the reflex of rats in the CPF group compared to those in the S/oil group at week 8. Although there was no significant change ($P < 0.05$) in the reflex righting time of rats in between the groups at week 16, the time to right the reflex of rats in the CPF group was comparatively higher relative to that in S/oil, VC or VC+CPF group. The present study has also demonstrated impairment of surface righting reflex in rats chronically exposed to CPF only. The increased reflex righting time observed in the CPF group reflects impairment in neuromuscular coordination and sensorimotor reflex. This finding agreed with that observed by Dam *et al.*^[49] where impairment of righting reflex was recorded in neonatal rats exposed to CPF. The deficit in righting reflex in the CPF group may be due to interference with ACh metabolism arising from AChE inhibition. Similarly, lipoperoxidative damage to the muscle may have contributed to the sensorimotor deficits in the CPF group. Vitamin C pretreatment was shown to have shortened the reflex righting time. This may be due to improvement in AChE activity, which eventually restored neuromuscular activity. Furthermore, the decreased lipoperoxidative damage to the muscle may have contributed to the amelioration of the CPF-evoked sensorimotor performance deficit.

Effect of various treatments on excitability scores

The effect of various treatments on excitability score is shown in Figure 7. There was a progressive decline in the excitability score of rats in the CPF and VC+CPF groups throughout the evaluation period, although the magnitude of the decline was higher in the former. Specifically, there

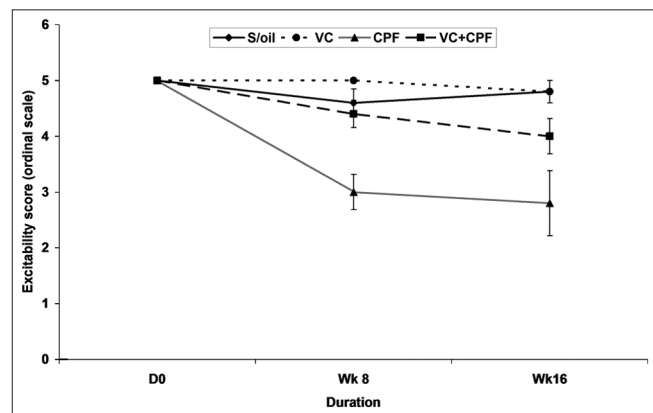


Figure 7: Effect of chronic exposure of Wistar rats to soya oil (S/oil), chlorpyrifos (CPF), and/or vitamin C (VC) on excitability scores dynamics ($n=5$ animals per group)

was a significant ($P < 0.05$) decline in the excitability score at weeks 8 and 16 when respectively compared to day 0 in the CPF group. No significant ($P > 0.05$) difference in the excitability score was recorded when the score of week 8 was compared to that of week 16 in the CPF group. The excitability score in the VC+CPF group was not significantly different at day 0 compared to that at week 8 or that in between week 8 and 16. However, there was a significant ($P < 0.05$) decline in the excitability score when values recorded at week 16 was compared to that of day 0. There was no significant ($P > 0.05$) change in the excitability score of rats in either the S/oil or VC group when the value recorded at day 0 was compared to either those of week 8 or week 16.

There were no significant changes ($P > 0.05$) in the excitability score between the groups at day 0. At week 8, the excitability score of the CPF group was significantly lower ($P < 0.01$) compared to VC group but no significant change relative to that of the S/oil or VC+CPF group. The excitability score in the CPF group was significantly lower ($P < 0.05$) compared to those in S/oil or VC group but no significant change ($P > 0.05$) relative to those in the VC+CPF group. There was no significant change ($P > 0.05$) in the excitability score of the VC+CPF group compared to that of the S/oil or VC group.

The progressive decrease in the excitability score of animals dosed with CPF only reflected the state of physical and mental alertness of the animals, indicating poor sensorimotor reflex and neuromuscular coordination. This deficit may be due to impairment in AChE activity, hence neuronal activity in the nervous tissue^[8] and muscle. Pretreatment with vitamin C significantly improved the excitability score. This may be due to improvement in AChE activity in the brain^[8] and muscle following its inhibition by CPF, thereby aiding in the restoration of neuromuscular function. Besides, vitamin C has been shown to be involved in the synthesis of neurotransmitter such as norepinephrine, which may increase brain excitability.^[50,51] Amelioration of CPF-induced lipoperoxidative damage to the brain^[8] and muscle by vitamin C may have complemented the improvement in excitability scores. The present finding corroborates that obtained by Ayo *et al.*,^[26] who showed the ability of vitamin C to mitigate low excitability score induced by road transportation stress in goats.

CONCLUSION

The dose of CPF used in the present was slightly higher than the 10 mg/kg low observable adverse effect level for 51-61% inhibition of brain cholinesterase activity^[52,53] and may therefore be described as relatively strong, although it constitute only 12.5% of the LD₅₀. This may explain the obvious alterations in motor function and sensorimotor

reflex in the CPF group. The lipoperoxidative changes induced by CPF may be partly central to the neurobehavioral aberrations recorded in the CPF group since it has been shown to alter the activities of membrane enzymes including AChE and affect the glycogen reserves. That also explains why pretreatment with vitamin C mitigates but not completely abolishes these neurobehavioral deficits.

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