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Research Article

Vitamin-C Attenuates the Neurobehavioural and Motor Dysfunctions Induced by Chlorpyrifos Acute Exposures

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ABSTRACT

Background: Chlorpyrifos is a toxic organophosphate pesticide commonly used for pest control to which humans are exposed. We investigated the anxiogenic and motor effects of the acute inhalational exposure to chlorpyrifos in mice as a predictor for human effects. The role of vitamin C in attenuating its toxicity was also investigated.

Methods: The Elevated Plus Maze (EPM) and Y-Maze (YM) models were used for assessing effects in relation to anxiety. For motor functions, the chimney, traction, climbing, inclined screen and swimming endurance tests were employed. The treatment schedule was: Group 1: Water spray (10 Puffs) only as control; Group 2: Vitamin C (100mg/kg; p.o.) 1h before chlorpyrifos (20 mg/m^3); Group 3:Water spray 1h before chlorpyrifos spray (20 mg/m^3); Group 4: Vitamin C (100 mg/kg; p.o.) only; Group 5: Diazepam (1mg/kg; i.p.) 30 min. before vitamin C (100mg/kg; p.o.).

Results: Chlorpyrifos treatment caused significant ($P<0.05$) reduction in time spent in the open arms of the EPM and YM, but Vitamin C treatment caused significant increases in time spent in the open arms of the EPM and YM. Also, in the chimney, traction, climbing, inclined screen and swimming endurance tests, chlorpyrifos caused significant increases in reaction times in the models. Conversely, vitamin C caused significant decreases in reaction times in all the models.

Conclusion: Acute exposure to chlorpyrifos through inhalation induced anxiety and impaired motor function. These effects were attenuated with vitamin C.

INTRODUCTION

Organophosphate pesticides (OPs) are among the most widely used chemicals for pest control. They are applied on agricultural food crops and animals, as well as in and around buildings, to kill a number of pests, including insects.[1] These OPs are environmental toxicants, which everyone is exposed to at considerable concentrations via various routes, including oral ingestion, dermal and inhalational routes. In addition to agricultural and domestic applications, OPs such as chlorpyrifos passes via air drift or surface run-off into surrounding waters and gets accumulated in different aquatic organisms, particularly fish, adversely affecting them.[2] This can cause acute poisoning and well-known symptoms including myosis, increased urination, diarrhoea, diaphoresis, lacrimation and salivation.[3]

Children particularly have been more susceptible to the health effects due to exposure to OPs. Effects like changes in children's cognitive, behavioral, and motor performance have been reported.[4] Exposure to OPs has been reported to affect sensorimotor performances in both the young[5-6] and adult[7-8] humans and animals.

Chlorpyrifos (O,O-diethyl-O-3,5,6-trichlor-2-pyridyl phosphorothioate) is a broad spectrum organophosphate insecticide. Some of its indoor uses has been banned by US Environmental Protection Agency in 2000 due to its neurotoxic effect, especially in children. Despite this restriction, chlorpyrifos remains a popular insecticide throughout the world.[9] The mechanism through which chlorpyrifos, and other organophosphate pesticides act is thought to be by interfering with signaling from the neurotransmitter acetylcholine to the enzyme acetylcholinesterase, thereby preventing this enzyme from deactivating acetylcholine in the synapse.[10-11] Specifically, the mechanism of chlorpyrifos-induced neurotoxicity is widely thought to be by inhibition of acetylcholinesterase (AChE) in the cholinergic neurons. This leads to accumulation of the neurotransmitter, ACh, in the central and peripheral nervous systems, eventually resulting

in overstimulation of muscarinic, nicotinic, and central cholinergic receptors.[12]

There is growing concern that chronic or subchronic low-level exposure to OPs cause neurobehavioral disorders such as autism, anxiety, depression, and attention deficit hyperactivity disorder [13-16] even in the living rodent brain.[17] All of these, therefore indicate that the pesticide may be involved in some neurodegenerative disorders [18] at doses that do not inhibit AChE,[19] the endocannabinoid system,[20] and oxidative stress.[8,19,21-23]

Oxidative stress has been implicated in the pathogenesis of many neurological and neurodegenerative diseases.[24,25-26]

Vitamin C is the most widely available water soluble antioxidant molecule that has shown tremendous promise in attenuating OP-induced health issues.[27-29] 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.[30] The usefulness of vitamin C is even currently highlighted, in the prevention and amelioration of various diseases that induce massive oxidative stress, such as corona virus diseases (e.g. COVID-19), though, yet undocumented. Currently, chlorpyrifos health risks are thought to be massive on the central nervous system.[31]

A previous study attempted to study the effect of vitamin C on chronic chlorpyrifos administration on muscular functions, however no reliable studies were found on the core models of anxiety and musculo-skeletal functions. Moreover, researchers have not focused on the acute effects of chlorpyrifos on neurobehavioral functions such as anxiety and muscle function.[32]

Therefore, this study was designed to assess the neurobehavioral effects induced by acute exposure to chlorpyrifos including behaviors such as anxiety and muscle weakness in juvenile animals as a predictive model for humans, and to investigate the role of antioxidant vitamin C in ameliorating these toxicities. Based on epidemiological and experimental animal studies which suggest that infants and children are more susceptible than adults to effects from low exposure to chlorpyrifos,[33-34] young mice were used in this study.

MATERIALS AND METHODS

Animals

Four to five weeks old, juvenile albino mice of either sex (12-13 g) used in this study were purposely bred in the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. The animals were maintained under standard environmental conditions (23-25°C, 12h/12h light/dark cycle). They were fed with growers' mash (Animal Care Limited, Abeokuta, Nigeria) and had access to drinking water ad libitum. Mice were acclimatized for 2 weeks prior to commencement of experiment procedures, which were in accordance with the provisions of the Experimentation Ethics Committee on Animal Use of College of Medicine, University of Lagos, Nigeria and the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals. [35]

Chemicals, Drugs

Chlorpyrifos (Termex® 480 grams/L, Dow Agro Sciences LLC, U.K.) concentrate was purchased from a local chemical store in Mushin, Lagos. It was diluted in distilled water to make a hydrous solution of 10% chlorpyrifos. The stock preparation was transferred to a tightly covered brown bottle and kept in a cupboard. On each day of experiment, 100 mL of the stock solution was poured into the sprayer. One puff of spray in the glass chamber was equivalent to 2 mg of chlorpyrifos per cubic meter (m3) for a 2-minute inhalation exposure, according to the United States Environmental Protection Agency.[1]

Diazepam injection (Roche®, U.K.)and vitamin C (Emzor Pharmaceuticals, Lagos, Nigeria) tablets were purchased at a local pharmacy in Ikeja, Lagos, Nigeria; insecticide aerosol sprayer was also purchased in Mushin, Lagos, Nigeria.

Elevated Plus Maze (EPM) Test

The elevated plus maze is used to examine anxiolytic activities in rodents.[36-37] The maze (30 cm × 6 cm × 6 cm, each arm) made of wood consist of two open and two closed arms across, at 60cm above ground level [38-39]. Five groups of five mice each were treated with the following schedule - Group 1: Water spray (10 Puffs) only as control; Group 2: Vitamin C (100 mg/kg; p.o.) 1h before chlorpyrifos (20 mg/m^3); Group 3: Water spray (10 Puffs) 1h before chlorpyrifos spray (20 mg/m^3); Group 4: Vitamin C (100 mg/kg p.o.) only; Group 5: Diazepam (1 mg/kg; i.p.) 30 min. before vitamin C (100 mg/kg; p.o.). Groups 2, 4 and 5 were pretreated with vitamin C for one hour, with Diazepam administered within 30 minutes of vitamin C administration for Group 5.One hour post-treatment, mice in Groups 4 and 5 were placed individually in the center of the maze, head facing towards the open arm while mice in Groups 1, 2 & 3 were sprayed with water and chlorpyrifos in a rectangular plexi-glass container covered, with little space left for aeration. The exposure time was two minutes before being placed individually in the center of the maze with head facing towards the open arm.

The experiment was performed using a video tracking system comprising a camera (Logitech C270, Lancashire UK) connected to a laptop using the Any-Maze Behavioral Tracking System (Stoelting Co., Illinois, USA). The duration of time spent and number of entries were captured within 5 min for each mouse (trial). The maze was wiped clean with 70% ethyl alcohol and dried after each trial. The percentage of time spent in the open arm and the percentage of open arm entries were taken as a measure of anxiety.

Y-Maze Test

The Y-maze is a model used to examine anxiolytic activities in rodents.[38,40] Five groups of five mice each were treated as mentioned in the EPM model. The exposure time was two minutes before being placed individually in the centre of a Y-shaped wooden maze (70cm × 15cm × 12cm). The average time each animal spends in each arm were recorded and expressed as percentage (open arm duration/total duration ×100).[38,40] The maze was wiped

clean with 70% ethyl alcohol and dried after each trial. The percentage of time spent in the open arm and the percentage of open arm entries were used as a measure of anxiety.

Chimney Test

Chimney test is used to investigate the muscle strength of mice. Each mouse was introduced at one end and allowed to move to the other end of a Pyrex glass tube (30 cm long × 3.0 cm diameter) marked at 20cm from base. When the animal reaches the 20cm mark, the tube was moved to a vertical position and immediately, the mouse tried to climb the tube with a backward movement.[41] The mice that successfully reached the mark within 30 seconds, were selected for further testing in four groups of five mice each. The test was repeated with screened animals as follows: Group 1: Water spray (10 puffs) only as control; Group 2: Vitamin C (100 mg/kg; p.o.) 1 h before chlorpyrifos (20 mg/m^3); Group 3: Water spray (10 puffs) 1 h before chlorpyrifos spray (20 mg/m^3); Group 4: Vitamin C (100 mg/kg p.o.) only. One hour post-treatment, mice in groups 4 were placed individually in the center of the maze, head facing towards the open arm while mice in Groups 1, 2 and 3 were sprayed with water and chlorpyrifos in a rectangular plexi-glass container covered, with little space left for aeration for two minutes. Thereafter, the test was carried out on each mice immediately. Percentage of mice showing no reaction based being able to perform backward movement in the glass tube were recorded.

Traction Test

Traction test is also used to investigate the muscle strength of mice. The fore paws of a mouse were placed on a small twisted wire rigidly supported above a laboratory bench top. Normal mice grasped the wire with the fore paws and when allowed to hang free, placed at least one hind foot on the wire within 5 seconds. Inability to place at least one hind foot marked failure in the traction test.[42] Previously screened mice were selected for further testing in four groups of five mice each. The test was repeated with screened animals as follows - Group 1: Water spray (10 puffs) only as control; Group 2: Vitamin C (100 mg/kg; p.o.) 1 h before chlorpyrifos (20 mg/m^3); Group 3: Water spray (10 puffs) 1 h before chlorpyrifos spray (20 mg/m^3); Group 4: Vitamin C (100 mg/kg p.o.) only. One hour post-treatment, mice in Group 4 were placed individually in the center of the maze, head facing towards the open arm while mice in Groups 1, 2 and 3 were sprayed with water and or chlorpyrifos in a rectangular plexi-glass container covered, with little space left for aeration for two minutes. Thereafter, the test was carried out on each mice immediately. Percentage mice showing negative response through placement of hind foot on the twisted wire was recorded.

Climbing Test

Mice were trained to climb a chain, 50 cm long by placing the fore paws of each animal on the free end of the chain.[38] The chain was suspended from a clamp standing on a laboratory bench (90 cm from ground). A normal mouse grasped the chain with the fore paws and when allowed to hang free, placed the two feet on the chain and climbed till it got to a marked point 2cm to the top of the chain. Mice that got to the mark within 30 seconds were selected for further tests. Previously screened mice were selected for further testing in four groups of five mice each. The test was repeated with screened animals as follows: Group 1: Water spray (10 puffs) only as control; Group 2: Vitamin C (100 mg/kg; p.o.) 1 h before chlorpyrifos (10 mg/m^3); Group 3: Water spray (10 puffs) 1 h before chlorpyrifos spray (10 mg/m^3); Group 4: Vitamin C (100 mg/kg p.o.) only. One hour post-treatment, mice in Group 4 were placed individually on the chain, while mice in Groups 1, 2 and 3 were sprayed with water and or chlorpyrifos in a rectangular plexi-glass container covered, with little space left for aeration for two minutes following which the test was carried out on each mouse immediately. Mean time taken to climb a chain to the marked point was recorded.

Inclined Screen Test

Each mouse was left on flat, slippery, rectangular glass (42cm × 37cm) inclined at 30 degrees to the horizontal for 10 minutes.[43] The test was performed as follows: Group 1: Water spray (10 puffs) only as control; Group 2: Vitamin C (100 mg/kg; p.o.) 1h before chlorpyrifos (10 mg/m^3); Group 3: Water spray (10 puffs) 1 h before chlorpyrifos spray (10 mg/m^3); Group 4: vitamin C (100 mg/kg p.o.) only. One hour post-treatment, mice in Group 4 were placed on the inclined rectangular glass. Mice in Groups 1, 2 and 3 were sprayed with water and or chlorpyrifos in a rectangular plexi-glass container covered, with little space left for aeration for two minutes, after which the test was carried out on each mouse immediately. Mean time taken to slide off the glass screen was recorded.

Swimming Endurance Test Coupled with Post-Swimming Inclined Screen Test

The model used was modified from Aluko *et al*.[44] The animals were divided into five groups of 5 animals each and were treated as follows: Group 1 - Water spray (10 puffs) only as control; Group 2: Vitamin C (100 mg/kg; p.o.) 1h before chlorpyrifos (20 mg/m^3); Group 3: Water spray (10 Puffs) 1h before chlorpyrifos (20 mg/m^3) spray; Group 4: Vitamin C (100 mg/kg p.o.) only; Group 5: Propranolol (40 mg/kg, i.m.). Groups 2, 4 were pretreated with Vitamin C, while Group 5 was pretreated with propranolol for one hour. Mice in Groups 1, 2 and 3 (one-hour post-treatment) were sprayed with water and or chlorpyrifos respectively in a rectangular plexi-glass container covered, with little space left for aeration for two minutes. Thereafter all groups of mice were made to swim in a plexi-glass water tank maintained at room temperature (30±2°C) until they sank. This was recorded as the swimming time. The animals were removed and allowed to recover and dry for about 5 min. The animals were subsequently tested for muscle coordination on an inclined screen 30° to the horizontal and the duration of stay until they slid off the screen was recorded.[43]

Statistical Analysis

The collected data for the models were expressed as mean ± S.E.M and analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Confidence interval was placed at 95%, such that in all cases, a value of $P < 0.05$ was considered significant. Statistical analysis was performed by GraphPad Prism software, version 6 (GraphPad Software, Inc., La Jolla, CA,

USA).

RESULTS

Elevated Plus Maze Test

Mice administered chlorpyrifos showed significant (*p<0.05) decreases in the mean time spent in the open arms relative to water spray (control) mice. Vitamin C however, caused mice to spend a significantly longer time in the open arms compared to the chlorpyrifos group. Combination of diazepam and vitamin C produced similar results (Fig. 1).

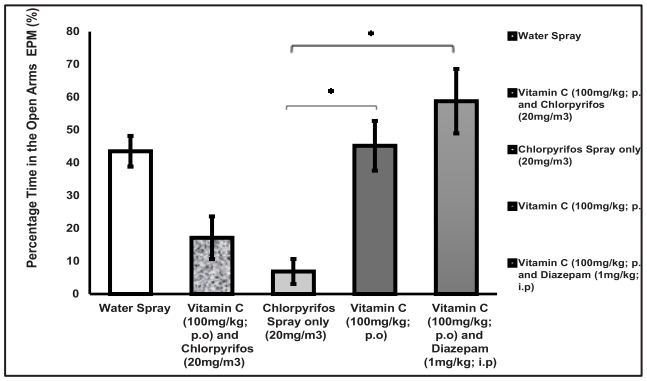

Figure 1: Effects of chlorpyrifos (20 mg/ m^3), vit. C (100 mg/kg) + chlorpyrifos (20 mg/ m^3) as well as vit. C (100 mg/kg) + diazepam (1mg/kg) on the percentage time spent in open arms in EPM. Values are expressed as mean ± SEM; n = 5 in each group. *P< 0.05, Chlorpyrifos versus Vit. C; *P< 0.05, chlorpyrifos versus vit. C and diazepam for percentage time spent in open arms using one-way ANOVA followed by Tukey's multiple comparison test.

Y-Maze Test

Chlorpyrifos treated mice produced significant (p<0.05) decreases in the mean time spent in the open arms compared with the control (water spray) mice. Vitamin C however, caused mice to spend a significantly longer time in the open arms compared to the chlorpyrifos group. Furthermore, vitamin c treatment combined with chlorpyrifos increased the time spent in open arms of the Y Maze. Combination of diazepam and vitamin C produced a significant increase in time spent in the open arm relative to total time in both arms (Fig. 2).

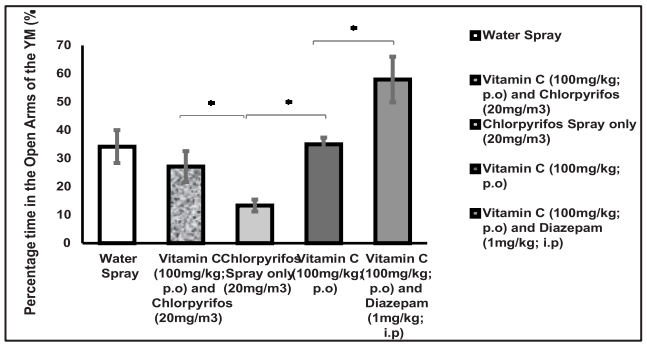

Figure 2: Effects of chlorpyrifos (20 mg/m^3),vit. C (100 mg/kg) + Chlorpyrifos (20 mg/m^3) as well as Vit. C (100 mg/kg) + Diazepam (1mg/kg) on the percentage time spent in open arms in Y-maze. Values are expressed as mean ± SEM; n = 5 in each group. * P< 0.05, chlorpyrifos versus vit. C; *P< 0.05 chlorpyrifos versus vit. C and diazepam for percentage time spent in open arm using one way ANOVA followed by Tukey's multiple comparison test.

Chimney Test

Chlorpyrifos produced a significant (P<0.05) negative response (100%) in mice when compared to control (water spray). Vitamin C (100 mg/kg) pretreatment caused a significant (up to 40%) reduction in negative response with 20 mg/m^3, chlorpyrifos. Control and vitamin C-only treated animals showed 0% negative responses (Fig. 3).

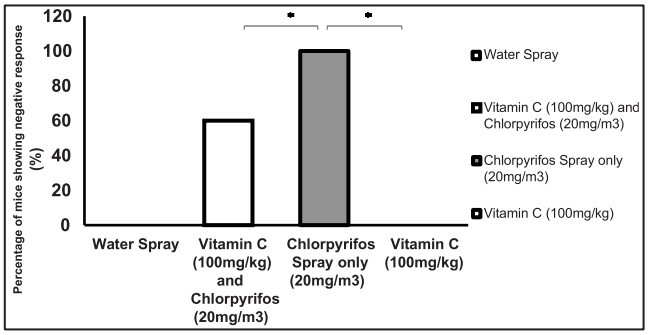

Figure 3: Effect of chlorpyrifos (20 mg/ m^3) and vitamin C (100mg/kg) + chlorpyrifos (20 mg/ m^3) on percentage mice showing negative response in chimney test. Values are expressed as percentages; n = 5 in each group. * P< 0.05, chlorpyrifos (20 mg/ m^3) versus vitamin C (100mg/kg) + chlorpyrifos (20 mg/ m^3). *P< 0.05 chlorpyrifos (20 mg/ m^3) versus vitamin C group for percentage mice showing negative using one way ANOVA followed by Tukey's multiple comparison test.

Traction Test

Chlorpyrifos (20 mg/m^3) showed significant (40%) negative response, when compared to control (water spray), which showed 0% negative response. Vitamin C (100 mg/kg) when used alone, like water, caused a significant (0%) negative test. 20 mg/m^3 chlorpyrifos with vitamin C pretreatment reduced the percentage negative test from 40% to 20% when compared with chlorpyrifos only (Fig. 4).

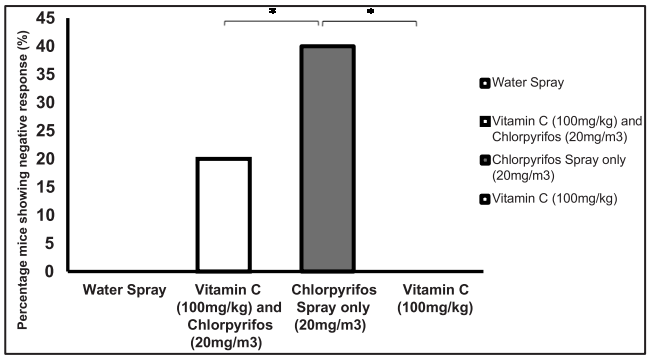

Figure 4: Effect of chlorpyrifos (20 mg/m^3) and vitamin C (100 mg/kg) on percentage mice showing negative response in traction test. Values are expressed as percentages; n = 5 in each group. * P< 0.05 Chlorpyrifos (20 mg/m^3) versus vitamin C. *P< 0.05 chlorpyrifos (20 mg/ m^3) versus vitamin C plus chlorpyrifos (20 mg/ m^3) group for percentage mice showing negative using one way ANOVA followed by Tukey's multiple comparison test.

Climbing Test

Chlorpyrifos (10 mg/m^3) showed significant increase in climbing times, when compared to water spray control. Vitamin C (100 mg/kg) when used alone, like water (control), produced significant reduction in climbing time. Moreover, vitamin C pretreatment with 10 mg/m^3 chlorpyrifos, caused a reduction in climbing times (Fig. 5).

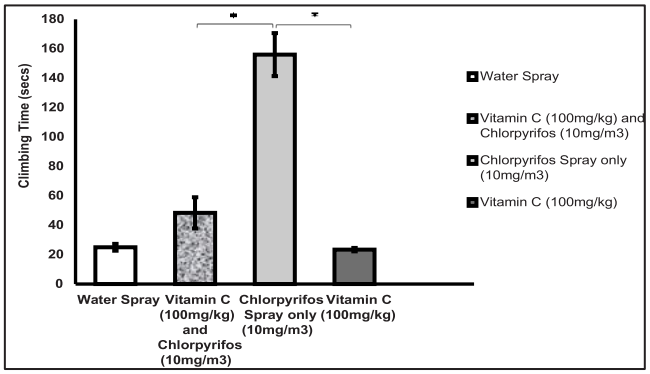

Figure 5: Effects of chlorpyrifos (10 mg/m^3) and vitamin C (100mg/kg) on the time taken to climb a chain in climbing test. Values are expressed as mean ± SEM; n = 5 in each group. *P< 0.05, chlorpyrifos (10 mg/m^3) versus vitamin C plus chlorpyrifos (10 mg/m^3) group. *P< 0.05,chlorpyrifos (10 mg/ m^3) only versus vitamin C only group using one way ANOVA followed by Tukey's multiple comparison test.

Inclined Screen Test

Chlorpyrifos (10 mg/m^3) showed significant reduction in time spent on the inclined screen. Vitamin C pretreatment did not produce significant changes in chlorpyrifos (10 mg/m^3) induced time spent in the inclined screen test (Fig. 6).

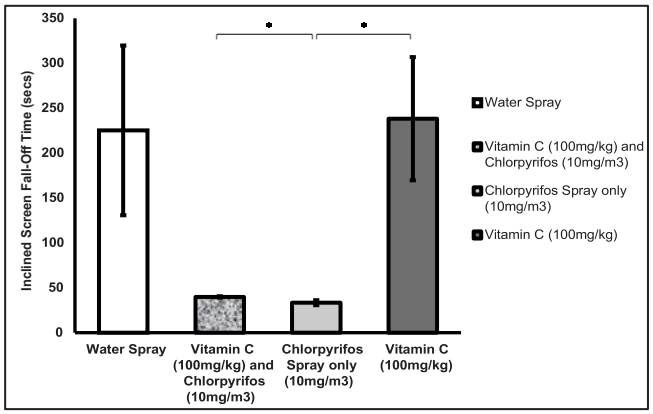

Figure 6: Effects of chlorpyrifos (10 mg/ m^3) and vitamin C (100 mg/kg) on the time taken to slide or fall-off the screen in inclined screen test. Values are expressed as mean ± SEM; n = 5 in each group. *P< 0.05, chlorpyrifos (10 mg/m^3) versus vitamin C plus chlorpyrifos (10 mg/m^3).*P< 0.05, chlorpyrifos (10 mg/m^3) versus vitamin C group for inclined screen fall-off time using one way ANOVA followed by Tukey's multiple

Swimming Endurance and Post-Swimming Motor Function - Inclined Screen Test

Chlorpyrifos (20 mg/m^3) showed significant reduction in swimming endurance time. Vitamin C pretreatment produced a significant increase in chlorpyrifos-induced reduction in the swimming time compared to chlorpyrifos only treatment (Fig. 7). In the post-swimming, inclined screen test, chlorpyrifos (20 mg/m^3) significantly (*P<0.05) decreased the mean time taken for mice to fall-off the screen when compared to controls; and vitamin C pretreatment caused an increase in time taken to fall-off the screen compared to chlorpyrifos only treatment. Propranolol increased the post -swimming fall off time from the inclined plane (Fig. 8).

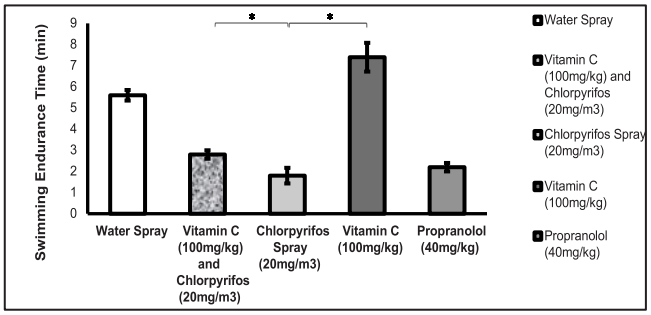

Figure 7: Effects of chlorpyrifos (20 mg/m^3) and vitamin C (100 mg/kg) on the time to sink in swimming endurance test. Values are expressed as mean ± SEM; n = 5 in each group. *P< 0.05,chlorpyrifos (20 mg/m^3) versus vitamin C plus chlorpyrifos (20 mg/m^3) group. *P< 0.05, chlorpyrifos (20 mg/m^3) versus vitamin C group for swimming time using one way ANOVA followed by Tukey's multiple comparison test.

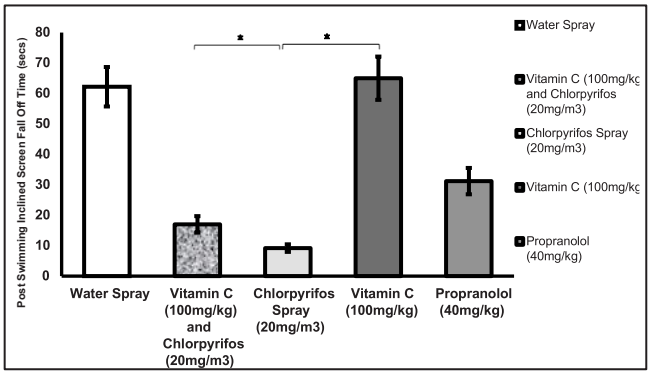

Figure 8: Effects of chlorpyrifos (20 mg/m^3) and vitamin C (100 mg/kg) on the time taken to slide or fall-off the screen in inclined screen test (post-swimming motor function test). Values are expressed as mean ± SEM; n = 5 in each group. *P< 0.05, chlorpyrifos (20 mg/m^3)versus vitamin C plus chlorpyrifos (20 mg/m^3) group. *P< 0.05, chlorpyrifos (20 mg/m^3) versus vitamin C group for inclined screen fall-off time using one way ANOVA followed by Tukey's multiple comparison test

DISCUSSION

This study investigated the neurobehavioural and motor effects of acute inhalational exposure to chlorpyrifos in mice. The model adopted for neurobehaviour was anxiety, while for the motor effect, musculoskeletal function models were used to assess the coordination related to muscle weakness or fatigue. In mice, when administered alone, acute inhalational exposure to chlorpyrifos produced a significant reduction of percentage time spent in open arms of the EPM and YM, compared with control, an indication of chlorpyrifos induced anxiogenic effects towards open and elevated area as is the manifestation of anxiogenic substances.[45] Contrarily, findings from a previous study by Chanda et al. showed that repeated low doses of chlorpyrifos during specific perinatal periods decreased anxiety-like behaviours.[46] However, while repeated oral dosing was used in that study, our findings suggest that acute, and perhaps, high dose acute inhalational exposure of chlorpyrifos to young mice increases anxiety, not otherwise..

The increases in percentage time spent in the open arms of the EPM and YM when Vitamin C was given prior to chlorpyrifos exposure is an indication for abolition of chlorpyrifos anxiogenic effect, therefore suggesting anxiolytic-like action by Vitamin C in both anxiety testing

models.[36-37] Various mechanisms have been ascribed to Vitamin C; but it is still unclear which of these is /are responsible for the anxiolytic-like actions observed in this research. One postulation is the antioxidant effect of vitamin C, which has been well documented.[52-54] 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.[24-26] Being a water soluble molecule, vitamin C can work both inside and outside the cells, and can neutralize free radicals and prevent free radical damage. This is a possible mechanism to consider for the anxiolytic-like finding in this study. Vitamin C is also an antioxidant that provides protection against some acute exogenous and chemical stressors such as hydroxyl radical, hydrogen peroxide (H_2O_2), and singlet oxygen[50-51] as presented by chlorpyrifos exposure. Therefore vitamin C confers its beneficial role in biological systems as an antioxidant and a reducing agent.[50, 56]

Another observation in this study is that there was a potentiation of the anxiolytic potential when vitamin C was combined with a standard anxiolytic drug, diazepam. This observation opens another opportunity for a possible role of vitamin C co-administration in drug potentiation and toxicities. Furthermore, vitamin C has been reported to be involved in the synthesis of neurotransmitter such as norepinephrine, which may increase brain excitability.[47-48] This is a possible strong mechanistic factor in the anxiolytic action shown by Vitamin C in this experiment.

In the muscular function models, acute inhalational exposure to chlorpyrifos also produced significant impairment in muscle coordination (muscle weakness) compared to the control mice as shown in the chimney, climbing and inclined screen tests. In all these models of muscle coordination, chlorpyrifos significantly impaired the muscle tone and function on mice. In support of these observations, symptoms of acute and chronic chlorpyrifos exposure documented in humans include muscle weakness and/or twitching.[49]. Vitamin C treatment, like that of the standard drug, diazepam, however, prevented these impairments. Among its many functions, vitamin C is an essential cofactor for several enzymes in the post-translational hydroxylation of collagen and biosynthesis of carnitine, conversion of the neurotransmitter dopamine to norepinephrine, peptide amidation, and in tyrosine metabolism. Supporting this assertions is a report that vitamin C is needed in the conversion of pro-collagen to collagen by oxidizing proline residues to hydroxyproline.[55] This is particularly important because collagen is an important component of the musculoskeletal system. All these biochemical reactions support skeletal muscle tone and functions. This could explain the improved muscle tone by vitamin C even in the presence of chlorpyrifos, as observed in this study.

In the swimming endurance test and post-swimming motor function assessment done via the inclined screen test, there was indication that chlorpyrifos significantly reduced the ability of mice to adapt to stressful situations as well as reduction in muscle coordination after undergoing stress compared to the control group.[44] Vitamin C and propranolol pretreatment significantly attenuated this chlorpyrifos induced stress. It might appear that chlorpyrifos produces its toxicity on muscle coordination or kinetics through a central action or inhibition of activity of central nervous system.

The ameliorative effect which Vitamin C showed in the various models used in this study could possibly be explained by acute oxidative protection against oxidative stress [52, 57] and free radicals in biological systems.[54] Research is ongoing in our laboratory to further ascertain the action.

The present findings corroborate those obtained by some researchers who showed the ability of vitamin C to mitigate low excitability score induced by road transportation stress in goats.[20]

CONCLUSION

Chlorpyrifos exposure induced toxic effects on behavioural and motor functions in mice as shown by its anxiogenic action as well as skeletal muscle dysfunctions in mice. Vitamin C ameliorated these dysfunctions caused by acute chlorpyrifos exposure. The mechanism of action of vitamin C could be attributable to its antioxidant effects.

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