Vitamin C attenuates short-term hematological and biochemical alterations induced by acute chlorpyrifos exposure in Wistar rats

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The present study was aimed at evaluating the effect acute chlorpyrifos exposure on short-term hematological and biochemical changes in Wistar rats, and the ameliorative effect of vitamin C. Four groups of 7 rats each were either given soya oil (2 ml/kg b.w.), CPF (42.5 mg/kg b.w.) and/or vitamin C (100 mg/kg b.w.). The rats were monitored for clinical signs and death over eight week period. Hematological evaluation revealed chlorpyrifos-induced alteration in packed cell volume, and levels of hemoglobin, red blood cells, absolute differential and total white blood cells count. Alteration in the levels of serum glucose, total proteins, albumin, globulin, electrolytes (Na⁺, K⁺, Cl⁻), urea, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and erythrocyte and liver malonaldehyde. Group pretreated with vitamin C mitigated the alterations in short-term hematologic and serum biochemical parameters induced by acute chlorpyrifos exposure. In conclusion, acute chlorpyrifos exposure has been shown by the present study to cause alteration in short-term hematological and biochemical parameters partly due to lipoperoxidative changes, which were ameliorated by vitamin C administration.

Keywords: Chlorpyrifos; acute exposure; short-term effect; hematology; clinical chemistry; lipoperoxidation; amelioration; vitamin C

INTRODUCTION

Several studies have revealed that single large dose exposure to organophosphate (OP) compounds including chlorpyrifos (CPF) results in long-term neurological impairments in humans (Duffy et al., 1979; Gershon and Shaw 1961; Kaplan et al., 1993; Metcalf and Holmes 1969; Rosenstock et al., 1991; Savage et al., 1988).

Studies using animal models have confirmed neurological sequelae due to acute OP exposure (Burchfield et al., 1976; Erich et al., 1993; Pope et al., 1992; Tandon et al., 1994) suggesting the persistence of acute OP effects (Duffy et al., 1979). Chlorpyrifos is one of the most widely used OP insecticides in the control of domestic and agricultural pests, despite the restriction placed on some of its domestic use by US Environmental Protection Agency in 2000. It is one of the most popular insecticides with long term neurological sequelae following acute exposure (Cañadas et al., 2005; Kaplan et al., 1993; Rosenstock et al., 1991). Like many other OP insecticides, its mechanism of acute toxicity is mainly due to inhibition of AChE. However, toxicity has been reported long after restitution of AChE activity. This has led to search for alternative mechanism of its toxicity. The induction of oxidative stress is one of the molecular

Abbreviations: CPF, chlorpyrifos; OP, organophosphate; AchE, acetylcholinesterase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; MDA, malonaldehyde; PCV, packed cell volume; RBC, red blood cells; Hb, hemoglobin; WBC, white blood cells.
mechanisms implicated in CPF-induced toxicity (Ambali et al., 2007; 2010 a and b; Gultekin et al., 2001; Vidyasagar et al., 2004). The long-term effect of acute CPF intoxication has centred mainly on neurological sequelae. There is a dearth of information on the effect of acute CPF exposure on both short- or long-term hematological and biochemical alterations and the effect of ascorbic acid. The present study therefore examines the effect of acute CPF exposure on short-term hematological and biochemical parameters, the role of lipoperoxidation and the ameliorative effect of vitamin C.

MATERIALS AND METHODS

Experimental animals

Twenty eight, twelve-weeks-old male Wistar rats weighing 173-178 g were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria. They were housed in metal cages and fed on standard rat pellets and water was provided ad-libitum. They were allowed to acclimatize for one week prior to the commencement of the experiment.

Chemical acquisition and preparation

Chlorpyrifos (TERMICOT®, Sabero Organics Ltd., Gujarat, India), was reconstituted in soya oil (Grand Cereal PLC, Jos, Nigeria) to 1%. Vitamin C (MED VIT C®, Dol-Med Laboratories Nigeria Ltd.) was reconstituted in distilled water to 100 mg/ml.

Experimental protocol

The twenty eight male rats were weighed and then divided at random into 4 groups of 7 animals in each group 12 hours prior to the commencement of the study. Group I (S/oil group) was given soya oil (2ml/kg) while group II (VC group) was administered vitamin C (100 mg/kg). Group III (CPF group) was administrated CPF (42.5mg/kg ~ 50% of LD50 of 85 mg/kg determined in a previous study (Ambali, 2009)). Group IV (VC+CPF group) was pretreated with vitamin C (100mg/kg) and then dosed with CPF (42.5mg/kg), 30 min later (Ambali et al., 2007). The regimens were administered once by oral gavage and the animals were then monitored for clinical signs and death over 8 week period. At the end of 8 weeks of monitoring, the animals were weighed and then sacrificed via jugular venesection after light ether anesthesia. Two millilitre of blood sample was collected from each animal into heparinized sample bottle for hematological analysis. Another set of 3 ml of blood was collected into test tube and then allowed to clot and then centrifuged at 800 x g for 10 min to obtain the serum, which was subsequently used for the biochemical assay. The study was carried out according to the specifications of the Ahmadu Bello University Animal Research Committee and in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 1996).

Hematological analysis

The hematological parameters evaluated using the method described by Dacie and Lewis (1991) were packed cell volume (PCV), haemoglobin (Hb) concentration, total erythrocyte (RBC), absolute and differential leucocytes (WBC) counts.

Serum biochemical analysis

Serum samples were evaluated for the level of glucose, total proteins, albumin and electrolytes (Na+, K+ and Cl-) urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) using an autoanalyzer (Bayer Clinical Chemistry Analyzer, Germany). Globulin was obtained by subtracting albumin concentration from the total serum protein.

Evaluation of erythrocyte and liver lipid peroxidation

The malonaldehyde (MDA) concentrations in the erythrocytes and the liver were evaluated as an index of lipid peroxidation using the double heating method of Draper and Hadley (1990).

Statistical analysis

Values expressed as mean±SEM were subjected to one-way analysis of variance (ANOVA) followed by Tukey’s test. The body weight of animals in each group at termination was compared with those obtained at the commencement of the study using the student t test. Values of P<0.05 were considered significant.

RESULTS

Clinical signs

Animals in the S/oil and VC groups did not show any apparent sign of toxicity. Toxic signs observed in the CPF group included restlessness, diarrhea, conjunctivitis, tremor, arched back, rough hair coat, huddling, and death was recorded (2 animals). Rats in the VC+CPF group showed mild restlessness and conjunctivitis.

Effect of treatments on body weight changes

The effect of the treatments on body weight changes is shown in Figure 1. There was a significant increase (P < 0.05) in the body weight gain at termination compared to those obtained at the commencement of the study in all the groups. However, rats in CPF group showed the least increase (7%) in body weight changes when compared to those recorded for S/oil (26%), VC (25%) and VC+CPF (15%) groups, respectively.

Effect of treatments on hematological parameters

Effect of treatments on packed cell volume

The effect of treatments on PCV is shown in Figure 2. The PCV in all the groups were not significantly different from each other. However, rats in the CPF group had 9.4% decrease in their PCV compared to the S/oil group.
**Figure 1.** Effect of acute exposure to soya oil, vitamin C and chlorpyrifos on short-term body weight changes in Wistar rats

**Figure 2.** Effect of acute exposure to soya oil, vitamin C and chlorpyrifos on short-term changes in packed cell volume in Wistar rats
Effect of treatments on hemoglobin concentration

There was no significant difference in the Hb concentration of rats in all the groups. However, rats in the CPF group showed a 10% drop in Hb concentration compared to 2.1% decrease recorded in the VC+CPF group, relative to the S/oil group (Figure 3).

Effect of treatments on red blood cell counts

The RBC counts were not significantly different in all the groups. However, the RBC count in the CPF group was lowered by 11%, compared to those recorded in the VC+CPF (2.6% drop) relative to the S/oil group (Figure 4).

Effect of treatments on white blood cell counts

There were no significant changes in the total WBC counts in rats from all the groups. However, rats in the CPF group had a 7.8% increase in WBC concentration relative to the S/oil group. On the other hand, the VC+CPF group had a 14.1% decrease in WBC concentration compared to the S/oil group (Figure 5).

Effect of treatments on serum biochemical parameters

Effect of treatments on glucose concentration

The glucose concentrations between the groups were not significantly different. However, a 19% decrease in glucose concentration was recorded in the CPF group while 0.8% increase in glucose concentration was observed in the VC+CPF group compared to the S/oil group (Figure 6).

Effect of treatments on total proteins, albumin and globulin concentrations

There were no significant changes in total serum proteins in all the groups. The serum proteins in the CPF and VC+CPF groups decreased by 2.8% and 0.1%, respectively, compared to the S/oil group (Figure 7). There were no significant changes in the albumin concentration in all the groups. However, the albumin
**Figure 4.** Effect of acute exposure to soya oil, vitamin C and chlorpyrifos on short-term changes in red blood cell concentration in Wistar rats

**Figure 5.** Effect of acute exposure to soya oil, vitamin C and chlorpyrifos on short-term changes in white blood cell concentrations in Wistar rats
Figure 6. Effect of acute exposure to soya oil, vitamin C and chlorpyrifos on short-term changes in glucose concentration in Wistar rats.

Figure 7. Effect of acute exposure to soya oil, vitamin C and chlorpyrifos on short-term alterations in total proteins, albumin and globulin concentrations in Wistar rats. *p<0.05 versus soya oil group.
Effect of acute exposure to soya oil, vitamin C and chlorpyrifos on short-term changes in serum electrolytes concentration in Wistar rats

Effect of treatments on serum electrolyte concentrations

There were no significant changes in the Na$^+$ concentration between the groups. However, the Na$^+$ concentration slightly increased in the CPF group by 1% compared to the S/oil group. The Na$^+$ concentration in the VC+CPF group showed a mild decrease (0.4%) relative to the S/oil group (Figure 8).

The differences in the K$^+$ concentration between the groups were not significant. However, the K$^+$ concentration was higher in the CPF (11%) and VC+CPF (4%) groups, respectively, relative to the S/oil group. There were no significant changes in the Cl$^-$ concentration between the groups. The Cl$^-$ concentrations in the CPF and VC+CPF group comparatively increased by 1.6% and 1.4%, respectively, relative to the S/oil group (Figure 8).

Effect of treatments on serum urea concentration

There were no significant changes in the urea concentration in between the groups. However, the urea concentration in the CPF group was comparatively higher (2.7%) relative to the S/oil group. The urea concentration in the VC+CPF group decreased by 8.3% relative to the S/oil group (Figure 9).

Effect of treatments on serum enzymes

The levels of AST were not significantly different between the groups. However, the level of AST was higher in the CPF (20%) and VC+CPF (6%) groups, respectively, relative to the S/oil group. The ALT activity was significantly reduced (P < 0.05; 39%) in the CPF group compared to those in the S/oil group. There were no significant changes in the levels of ALT between the other groups. However, the activity of ALT also decreased by 25% in the VC+CPF group compared to the S/oil group. There were no significant changes in the levels of ALP between the groups. However, the ALP activity in CPF group increased by 8.2% compared to the
S/oil group. The ALP activity was comparatively higher (3.6%) in the VC+CPF group relative to the S/oil group (Figure 10).

**Effect of treatments on erythrocyte and liver lipoperoxidation**

There was a significant increase (p<0.05) in the erythrocyte MDA concentrations in the CPF group compared to S/oil and the VC groups, respectively. The erythrocyte MDA concentration in both the CPF and VC+CPF groups increased by 28% and 13%, respectively, compared to the S/oil group (Figure 11). The liver MDA concentrations were not significantly different between the groups. However, the MDA concentration in the CPF and VC+CPF groups increased by 17% and 8%, respectively, relative to the S/oil group (Figure 12).

**DISCUSSION**

The clinical signs observed in the rats exposed to CPF only were consistent with cholinergic symptoms associated with cholinesterase inhibition (Eaton et al., 2008). The cholinergic signs were more severe in the CPF-treated group (which resulted in two deaths) compared to group pretreated with vitamin C. This revealed the ability of the vitamin to mitigate toxic signs induced by CPF. Similar results have been observed in repeated CPF toxicity studies (Ambali et al., 2007, 2010a and b; Ambali, 2009). The reduction in severity of signs of toxicity in rats pretreated with vitamin C probably revealed the role of oxidative stress in the short-term toxicity induced by acute CPF exposure in rats. Besides, vitamin C has been shown to increase the activity of paraoxonase (Jarvik et al., 2002), which is essential in the detoxification of OP and also aids in the reactivation of AChE (Yavuz et al., 2004). In addition, vitamin C is a cofactor in many enzymatic reactions, which might aid the body to withstand CPF-induced stress. All these factors may have accounted for the mild toxicosis observed in the vitamin C pretreated group.

The present study has also shown that acute exposure to large dose CPF adversely affects body weight gain. Although, there were significant increases in the body weight gain of all the groups, a comparatively lower body weight gain was however, recorded in the CPF group. The adverse effect may be due to induction of lipid peroxidation by CPF, which reduced the body tissue mass (Rowlands et al., 2000) possibly via deteriorative changes in the fat and protein contents. In addition, CPF-oxon, the toxic metabolite of CPF has been shown to inhibit the activity of cholesteryl ester hydrolase (Civen et al., 1977), thereby reducing the ability of the CPF-treated animals to cope with stress. Besides, the severe cholinergic signs in the group exposed to CPF only further increased the stress on the animals. Therefore, the lowered body weight gain in rats exposed to CPF only...
Figure 10. Effect of acute exposure to soya oil, vitamin C and chlorpyrifos on short-term changes in serum liver enzymes in Wistar rats. *p<0.05 versus soya oil group.

Figure 11. Effect of acute exposure to soya oil, vitamin C and chlorpyrifos on short-term changes in erythrocyte malonaldehyde concentration in Wistar rats
may be due to the combined effects of oxidative stress, adrenal-mediated stress and cholinergic stress. Pretreatment with vitamin C improved the weight gain of the animals by 15% compared to 6.7% obtained in those group exposed to CPF only. This demonstrated the role of oxidative stress in the CPF-induced adverse body weight changes.

Acute exposure to CPF has also been shown to cause short-term adversity on hematological parameters as revealed by a decrease in PCV, RBC and Hb concentration in rats exposed to the OP only. Previous studies have shown that repeated CPF exposure causes anemia in rats (Ambali, 2009; Ambali et al., 2010a; Goel et al., 2006) and fish (Ramesh and Saravanan, 2008). Therefore, the parameters indicative of anemia observed in rats exposed to CPF only may be due to the ability of the OP compound to decrease tissue iron concentration (Goel et al., 2006), interferes with Hb biosynthesis and induce RBC lifespan shortening (Ray, 1992) or even increase in erythrocyte fragility (Ambali et al., 2010ab). The high lipoperoxidative changes in the erythrocyte of rats exposed to CPF only as indicated by high MDA concentration demonstrates the role of oxidative stress in the anemia observed in the present study. Pretreatment with vitamin C improved the RBC parameters depressed by CPF. This may be due to the ability of the antioxidant vitamin to improve the absorption of iron from the gut (Iqbal et al., 2004; Wardlaw, 1999) by facilitating the reduction of oxidized iron to its reduced form (Sayers et al., 1973). Furthermore, the antioxidant effect of vitamin C may have improved the integrity of the RBC compromised by CPF-induced oxidative stress, as shown by a relatively lowered MDA concentration in the vitamin-pretreated group.

The study revealed an apparent increase in the WBC concentration in the group exposed to CPF only. The reason for the apparent leukocytosis is not known. This finding contradicted the result of previous study which showed that repeated CPF exposure causes leukopenia (Ambali et al., 2007, 2010a; Goel et al., 2006). The implication of the apparent leukocytosis is not known, especially as it relates to the immune system and deserves further studies. This is especially in the light of studies that have shown the ability of pesticides to be toxic to the immune cells via the induction of necrosis and apoptosis (Rabideau, 2001). Pretreatment with vitamin C did ameliorate the CPF-induced leukocytosis. The reason for the apparent restoration of vitamin C on CPF-evoked leukocytosis is not known for certain but may be due to its modulatory role on the immune cells.

The decrease in glucose concentration in the CPF group observed in the present study agreed with the findings Szabo et al. (1988). This however contraindicated the hyperglycemia earlier reported following repeated CPF (Ambali, 2009) and other OPs (reviewed by Rahimi and Abdollahi, 2007) exposure. The reason for the contradiction is not known for certain but may relate to CPF-induced hepatic damage, resulting in alteration in glucose metabolism. In addition, CPF has been shown to cause damage to the zona fasiculata of

![Figure 12. Effect of acute exposure to soya oil, vitamin C and chlorpyrifos on short-term changes in liver malonaldehyde concentration in Wistar rats](image-url)
the adrenal cortex (Barna-Lloyd et al., 1990; Yano et al., 2000), the region responsible for the production of glucocorticoid, a hormone that stimulates gluconeogenesis during stress. This effect on adrenal gland leads to decreased gluconeogenesis and hence low glucose concentration. Pretreatment with vitamin C ameliorated the CPF-induced changes in glucose level, indicating the role of oxidative stress in the hypoglycemic response. Vitamin C may have prevented oxidative damage to the liver and preserve the integrity of the adrenal cortex. The reduced TP observed in the CPF group agreed with the previous findings (Ambali, 2009; Szabo et al., 1988). The relatively lowered TP in the CPF group was apparently due to low globulin. This contradicts previous findings where CPF-induced hypoproteinemia has been attributed to hypalbuminemia (Ambali, 2009; Goel et al., 2006). Indeed, high albumin concentration was recorded in the group exposed to CPF in the present study. However, Szabo et al. (1988) observed hypoglobulinemia following repeated CPF exposure in rats. The reason for the apparent reduction in globulin concentration following acute CPF exposure is not known for certain, especially in the light of apparent leukocytosis observed in the CPF group. This may however be due to the ability of CPF to induce cytotoxic damage to the cells of the immune system via the induction of necrosis or apoptosis (Corcoran et al., 1994; Rabideau, 2001) resulting in the destruction of the source of these immunoglobulins. In addition, the increased hepatic MDA concentration in the CPF group, which is indicative of oxidative damage to the liver, the principal organ responsible for production of globulins (Ramesh and Saravanan, 2008) may have partly played a role in the reduced serum globulin. Furthermore, the increased tissue repair and detoxification mechanism during stress may have been partly responsible for the apparently lowered serum proteins (Neff, 1985) in the CPF group. On the other hand, pretreatment with vitamin C was shown to improve the total protein and the globulin concentrations. This shows that oxidative stress plays an important in the alteration of serum proteins concentration observed in CPF poisoning. The vitamin may have protected the liver and other cells of the immune system from CPF-induced oxidative damage. Besides, vitamin C stimulates the immune system by enhancing T-cell proliferation, which ultimately assists the B-cells to synthesize immunoglobulin (Campbell et al., 1999; Naidu, 2003).

The study has shown that single high dose level of CPF did not significantly alter the serum concentration of Na⁺, K⁺ and Cl⁻. This is in agreement with the findings of Ambali et al. (2007) and Ambali (2009). Of note however is that exposure to CPF was shown to cause about 11% increase in serum K⁺ concentration compared to the S/oil group. This shows that acute exposure to large dose CPF may have caused oxidative damage to the muscle. Pretreatment with vitamin C apparently restored the K⁺ concentration probably due to the reduced lipoperoxidative damage to the muscle.

The present study did record a slight (2.7%) increase in urea concentration in rats exposed to CPF only. This agrees with the previous findings in our laboratory (Ambali et al., 2007; Ambali, 2009). This apparent increase in urea revealed the ability of the acute large dose CPF exposure to cause short-term low-grade pathological changes in the kidneys. The apparent normalization of the urea concentration observed in vitamin C pretreated group revealed the role of oxidative stress in these pathological changes.

The significant decrease in the activity of ALT observed in the CPF group agreed with those recorded in previous studies (Ambali et al., 2007; Barna-Lloyd et al., 1990; Szabo et al., 1988). The reason for the low level of serum ALT is not known and besides, the toxicological significance of this remains obscure. However, pretreatment with vitamin C was shown by this study to partly restored the levels of ALT although is still relatively low compared to the soya oil group.

An apparent elevation (19.6%) in the AST activity of rats exposed to CPF only compared to those of the soya oil group indicates some level of damage to any or all of the organs producing this enzyme, such as the liver, skeletal and cardiac muscles. This finding agreed with those obtained in previous study (Ambali, 2009; Gomes et al., 1999; Yoshida et al., 1985;). Indeed, it has been revealed that CPF causes damage to the liver (Ambali, 2009; Goel et al., 2005) and the cardiac muscles (Ambali, 2009), probably through the induction of oxidative stress. Increased peroxidation can result in changes in cellular metabolism in the hepatic and extrahepatic tissue (Das et al., 2001). The oxidative damage induced by CPF may have been prevented by pretreatment with vitamin C, which apparently brought about restoration of the AST activity in rats in the vitamin C +CPF group.

Similarly, the apparent increase in serum ALP in rats exposed to CPF only agreed with previous works (Ambali et al., 2007; Ambali, 2009; Goel et al., 2005). This finding shows that acute CPF exposure causes short-term pathological lesions in any or all of the organs involved in the synthesis and/or release of ALP such as the liver, kidneys, bones, muscles and intestinal mucosa probably due to oxidative damage. The apparent restoration of the ALP activity in rats pretreated with vitamin C further underscore the role of oxidative damage in the apparent increase in ALP activity. Pretreatment with vitamin C apparently normalized the ALP activity by restoring it to almost the values obtained in the S/oil group.

The results from the present study strongly suggest that increased lipoperoxidative changes to the erythrocyte, liver and kidneys may have been partly responsible for the short-term alteration in hematological and biochemical parameters observed in group exposed to single large dose of CPF. The persistence of CPF-induced biochemical and hematological alterations may
be due to its lipophilic nature. CPF is reported to have an unusually long effect on AChE, apparently due to its unique lipophilicity, which enhances its storage in the tissue and their slow release into the blood stream (Chiappa et al., 1995). The persistence in the tissue may have continually triggered increased lipoperoxidative damage to the organs and tissue resulting in the short-term hematological and biochemical alterations. Lipid peroxidation is a free radical mediated process that involves the formation and propagation of lipid radicals, the uptake of oxygen and the rearrangement of the double and unsaturated lipids, resulting in a variety of degraded products that eventually cause destruction of membrane lipids (Dattani et al., 2010).

Although vitamin C was administered once during the study, its pretreatment was shown to have reduced or quenched any free radical that may have been triggered by the initial interaction of the CPF with the tissue. This helps the body overcome the adverse effect of oxidative damage to the tissues. Therefore, pretreatment with vitamin C has an ameliorating effect on CPF-induced clinical, hematologic and biochemical parameters. Vitamin C has been shown to reduce lipid peroxidation caused by toxic substances (Ambali et al., 2007; 2010a; Apperonth et al., 1997; Gultekin et al., 2000) by scavenging superoxides, hydroxyl radicals and various lipid hydroperoxides, in addition to restoring the antioxidant properties of vitamin E (Sadanan et al., 2008). Furthermore, this protective role exhibited by vitamin C may have stemmed from its other non-antioxidant roles. Vitamin C enhances the activity of paraoxonase I (Jarvik et al., 2002), resulting in the improvement in the detoxification of CPF-oxon, the active metabolite of CPF. Vitamin C has also been shown to enhance the restoration of AChE activity (Yavuz et al., 2004), thereby reducing the hematological and biochemical lesions associated with the OP exposure.

In conclusion, acute exposure of rats to CPF has been shown by the present study to cause short-term alterations in hematological and biochemical parameters, partly due to induction of lipoperoxidative changes and oxidative stress. Pretreatment with vitamin C did attenuate the CPF-induced hematological and biochemical alterations in Wistar rats partly due to its antioxidant effect.

References


