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Hematological changes induced by subchronic co-administration of chlorpyrifos and lead in Wistar rats: Alleviating effect of vitamin C

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ABSTRACT

Lead and chlorpyrifos (CPF) are among the most pervasive environmental contaminants that adversely affect human and animal health. Oxidative stress is a common molecular mechanism implicated in lead and CPF poisoning. The present study was aimed at evaluating the ameliorative effect of vitamin C on hematological changes induced by subchronic co-administration of CPF and lead in Wistar rats. Forty adult male Wistar rats were divided into 4 groups of 10 animals in each group. Group I was corn oil (2 ml/kg) while group II was administered vitamin C only (100 mg/kg). Group III was co-administered with CPF (4.25 mg/kg) and lead (250 mg/kg). The regimens were administered once daily by gavage for a period of nine weeks. The rats were sacrificed at the end of the dosing period and blood samples were analyzed for hematological parameters. The study revealed that alterations in pack cell volume, erythrocyte indices, and concentrations of hemoglobin, red blood cells, erythrocyte malonaldehyde, platelets, and absolute total and differential white blood cells induced by subchronic co-administration of CPF and lead were ameliorated by vitamin C. The attenuation of this hemotoxicity by vitamin C may be partly due to its antioxidant properties.

Key words- chlorpyrifos, lead, co-administration, hemotoxicity, oxidative stress, vitamin C.

INTRODUCTION

An increasing number of natural and man-made pollutants have pervaded the environment in the last few decades, which ultimately affect the health and well being living organisms. The need to improve food production in order to feed the ever-growing world population and the increasing industrialization and technological advancement have increased the environmental abundance of these contaminants. These pollutants interact with themselves and the environment to alter the health and well being of man and animals. Hitherto, the current understanding of the mechanisms

and effect of environmental chemicals and contaminants are based on studies conducted in laboratory animals using one agent at a time [1, 2]. However, the results of most of these studies may be misleading as they do not reflect the reality in the environment, since there are numerous contaminants interacting to alter physiological status of organisms.

Chemical pesticides and heavy metals are widely available in the environment. Chemical pesticides are one of the most widely available environmental contaminants that are deliberately released to the environment to improve the quantity and quality of food to feed the ever-increasing world human and animal population and promote public health. Organophosphate (OP) insecticides constitute one of the most popular pesticides, especially for agricultural and domestic purposes, accounting for about 50% of the global insecticidal use [3]. Chlorpyrifos (CPF), a broad spectrum OP insecticide that was introduced to the American market in 1965 is one of the most widely used OP insecticides in agriculture and public health [4, 5], despite restrictions placed on some of its residential uses by the United States Environmental Protection Agency in 2000. Lead, on the other hand, is one of the most pervasive heavy metal contaminants with widespread industrial and domestic applications. The metal has long been recognized as a poison to living organisms, with negative effects on general health, reproduction, behaviour, and under certain conditions can result in death [6]. Therefore, CPF and lead are among the most important environmental contaminants due to their widespread use, availability and pervasiveness in the environment.

Although the mechanisms of toxicity of CPF and lead are quite diverse, studies have shown that oxidative stress induction is a common biochemical mechanism involved in their toxicity. Oxidative stress, which results from the accumulation of free radicals and reactive oxygen species beyond the normal capacity of the body's antioxidant system to neutralize is known to cause organ and tissue damage. In this type of situation, the body must be supplemented with exogenous antioxidants to cope with the oxidative challenges. Vitamin C has been shown to mitigate toxicity induced by CPF [7, 8, 9] and Pb [10, 11]. The present study was aimed at evaluating the ameliorating effect of vitamin C on hemotoxicity induced by subchronic co-exposure to CPF and Pb in Wistar rats.

MATERIALS AND METHODS

Experimental animals

Forty 6-week old adult male Wistar rats weighing 132-146g were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The rats were fed on standard rat pellets and water was provided *ad libitum*. The experiment was performed in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) [12].

Chemicals

Commercial grade CPF (TERMICOT[®] 20% EC, Sabero Organics, Gujarat, India) was reconstituted in corn oil to make 10% stock solution, which was subsequently used for the experiment. Analytical grade lead acetate (Kiran Light Laboratories, Mumbai, India) used for the study was reconstituted into a 20% stock solution in distilled water. Commercial grade vitamin C

tablets (Emzor Pharmaceutical Ltd, Nigeria, BN: 618N) was prepared in distilled water to make 10% stock solution.

Animal treatments

Forty male Wistar rats were divided into four groups of 10 animals per group. Group I (C/oil) was administered corn oil (2 ml/kg), while group II (VC) was administered vitamin C (100 mg/kg). Group III (CPF+Pb) was co-administered CPF (4.25 mg/kg~1/20th LD₅₀ [13] and lead acetate (225 mg/kg~1/20th LD₅₀ [11]). Group IV (VC+CPF+Pb) was pretreated vitamin C and then co-administered with CPF (4.25 mg/kg) and Pb (225 mg/kg), 30 min later. These regimens were administered orally by gavage once daily for a period of 9 weeks. At the termination of the dosing, the animals were sacrificed via jugular venesection after light chloroform anesthesia. Blood samples collected into heparinized test tubes were examined for hematological parameters.

Hematological evaluation

Two milliliter of blood collected into heparinized sample bottles were analyzed for the levels of hematological parameters such as packed cell volume (PCV), hemoglobin (Hb), total red blood cells (RBC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cell (WBC) and total platelets count using an automatic hematological assay analyzer, Advia 60[®] Hematology system (Bayer Diagnostics Europe Ltd, Ireland). Blood smears were also stained with Giemsa for differential WBC count [14] while the neutrophil-lymphocyte ratio was calculated.

Evaluation of erythrocyte malonaldehyde concentration

Erythrocyte malonaldehyde (MDA) concentration, as a marker for lipid peroxidation, was determined by the double-heating method of Draper and Hadley [15] as modified by Altuntas et al. [16]. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex, $1.56 \times 10^5 \text{ cm}^{-1}\text{M}^{-1}$, and expressed in nanomoles per gram of hemoglobin.

Statistical Analysis

Data were expressed as mean \pm SEM and subjected to one-way analysis of variance followed by Tukey's multiple comparison test using Graph Pad Prism version 4.0. Values of $P < 0.05$ were considered significant. Where necessary, the difference in the mean value of the data obtained between each group was analyzed as %.

RESULTS

Effect of treatments on pack cell volume

The effect of treatments on PCV is shown in Figure 1. There was a significant ($P < 0.05$) decrease in the PCV in CPF+Pb group compared to C/oil ($P < 0.05$), VC ($P < 0.01$) or VC+CPF+Pb ($P < 0.05$) group. There was no significant ($P > 0.05$) difference in PCV in the VC+CPF+Pb group compared to C/oil or VC group (Table 1).

Effect of treatments on hemoglobin concentration

There was a significant decrease in the Hb concentration in the CPF+Pb group compared to C/oil ($P < 0.05$), VC ($P < 0.01$) or VC+CPF+Pb ($P < 0.05$) group. The Hb concentration in the

VC+CPF+Pb group was not significantly ($P>0.05$) different from those obtained in the C/oil or VC group (Table 1).

Effect of treatments on red blood cell concentration

The effect of treatments on RBC concentration is shown in Table 1. There was a significant ($P<0.05$) decrease in the RBC concentration in the CPF+Pb group compared to C/oil, VC or VC+CPF groups. There was no significant ($P>0.05$) change in the RBC count in the VC+CPF+Pb group compared to C/oil or VC group.

Effect of treatments on erythrocyte indices

The effect of treatments on erythrocyte indices is shown in Table 1. There was no significant change in the MCV in between the groups. The MCV in CPF+Pb group was comparatively higher compared to C/oil (2.9%), VC (2.4%) or VC+CPF+Pb (2.4%) group.

The MCH in the different groups were not significantly ($P>0.05$) different from each other. The MCH in the CPF+Pb group was comparatively lower compared to C/oil (0.9%) or VC (2.9%) group but was marginally higher (0.3%) compared to VC+CPF+Pb group.

The MCHC in the CPF+Pb group was not significantly different ($P>0.05$) compared to any of the groups but was comparatively lower compared to C/oil (4.3%), VC (1.5%) or VC+CPF +Pb (1.5%) group.

Effect of treatments on white blood cell count

The effect of treatments on absolute WBC count is shown in Table 2. There was no significant difference in the WBC count in between the groups. However, the WBC count in the CPF+Pb group was relatively lower compared to C/oil (11.7%), VC (11.7%) or VC+CPF+Pb (7%) group. The absolute differential leukocyte count revealed no significant ($P>0.05$) change in the neutrophil count in between the groups. However, the neutrophil count in the C/oil was comparatively higher relative to C/oil (46.7%) and VC (6.7%) groups but was relatively lower compared to VC+CPF+Pb group (0.7%) (Table 2)

The lymphocyte count in the C/oil group increased significantly ($P<0.05$) relative to the CPF+Pb or VC+CPF+Pb group. Although there was no significant change ($P>0.05$), the mean lymphocyte count in the C/oil group increased by 44% over those recorded in the VC group. The lymphocyte count in the VC+CPF+Pb group was not significantly different ($P>0.05$) compared to CPF+Pb group, however, the value recorded in the former group increased by 16% over those in the latter group (Table 2).

The monocyte and the eosinophil counts in between the groups were not significantly different. However, the eosinophil count in the CPF+Pb group was comparatively higher compared to the other groups (Table 2).

Effect of treatments on neutrophil:lymphocyte ratio

There was no significant ($P>0.05$) difference in the neutrophil:lymphocyte ratio (NLR) in between the groups, The NLR in the CPF+Pb group increased comparatively relative to C/oil (80%), VC (33%) or VC+CPF+Pb (7.1%) group (Table 2).

Effect of treatments on platelets count

There was a significant ($P < 0.05$) decrease in the platelet count of rats in the CPF+Pb group compared to C/oil or VC group. Although not significant ($P > 0.05$), the platelet count in the CPF+Pb group decreased by 9.5% compared to VC+CPF+Pb group. There was no significant ($P > 0.05$) change in the platelet count of VC+CPF+Pb group compared to C/oil or VC group (Figure 1)

Effect of treatments on erythrocyte malonaldehyde concentration

There was a significant increase in the MDA concentration in the CPF+Pb group compared to C/oil ($P < 0.01$), VC ($P < 0.01$) or VC+CPF+Pb ($P < 0.05$) group. There was a significant ($P < 0.05$) increase in the MDA concentration in the VC+CPF+Pb group compared to C/oil group but no significant change ($P > 0.05$) when compared to the VC group (Figure 2).

Table 1: Effect of chronic exposure to corn oil (C/oil), vitamin C (VC) and/or chlorpyrifos (CPF) on pack cell volume (PCV), red blood cell (RBC) and hemoglobin (Hb) concentrations, and erythrocyte indices in Wistar rats.

Parameters	C/oil	VC	CPF+Pb	VC+CPF+Pb
PCV (%)	39 ± 1.9	39 ± 3.2	25 ± 2.1 ^{acd}	37 ± 1.8
Hb concentration (g/dL)	13.2 ± 0.6	13.2 ± 1.1	8.3 ± 0.68 ^{acd}	12.3 ± 0.60
RBC count ($\times 10^{12}/L$)	6.6 ± 0.39	6.7 ± 0.62	4.2 ± 0.35 ^{abc}	6.2 ± 0.3 ^d
MCV (fL/cell)	58.7 ± 0.6	59 ± 1.0	58.8 ± 0.9	60 ± 0.03
MCH (pg/cell)	20 ± 0.3	20.4 ± 9.4	20 ± 0.1	20.2 ± 0.23
MCHC (g/dL)	34.3 ± 0.3	33.3 ± 0.3	32.8 ± 0.08	33.3 ± 0.05

^a $p < 0.05$ versus corn oil (C/oil) group; ^b $p < 0.05$ versus vitamin C (VC) group; ^c $p < 0.05$ versus vitamin C+chlorpyrifos group; ^d $p < 0.01$ versus vitamin C group. Values are mean ± SEM of 5 animals per group. NB- MCV- mean cell volume; MCH- Mean corpuscular hemoglobin; MCHC- Mean corpuscular hemoglobin concentration.

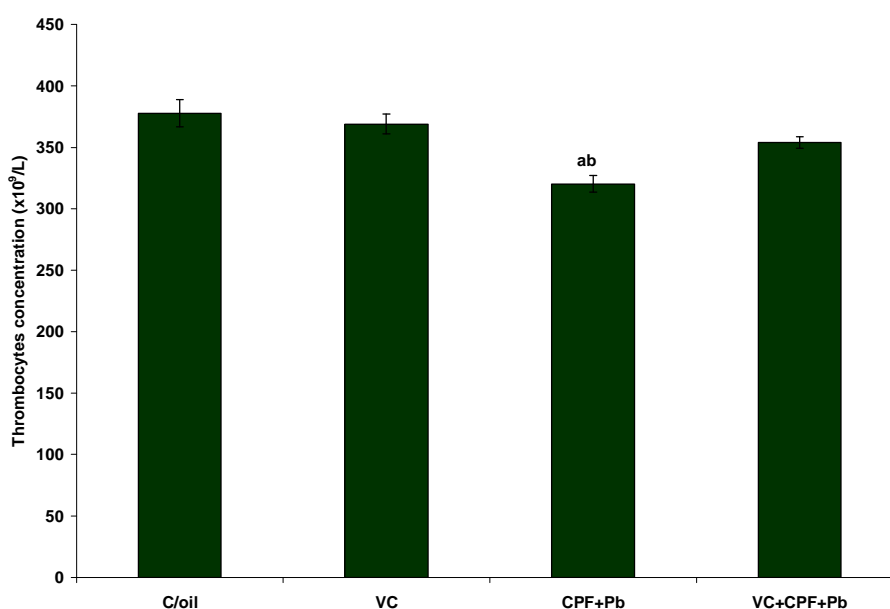


Figure 1: Effect of corn oil (C/oil), vitamin C (VC) and/or co-administration of chlorpyrifos (CPF) and lead (Pb) on total platelet count in Wistar rats. ^a $P < 0.05$ versus C/oil

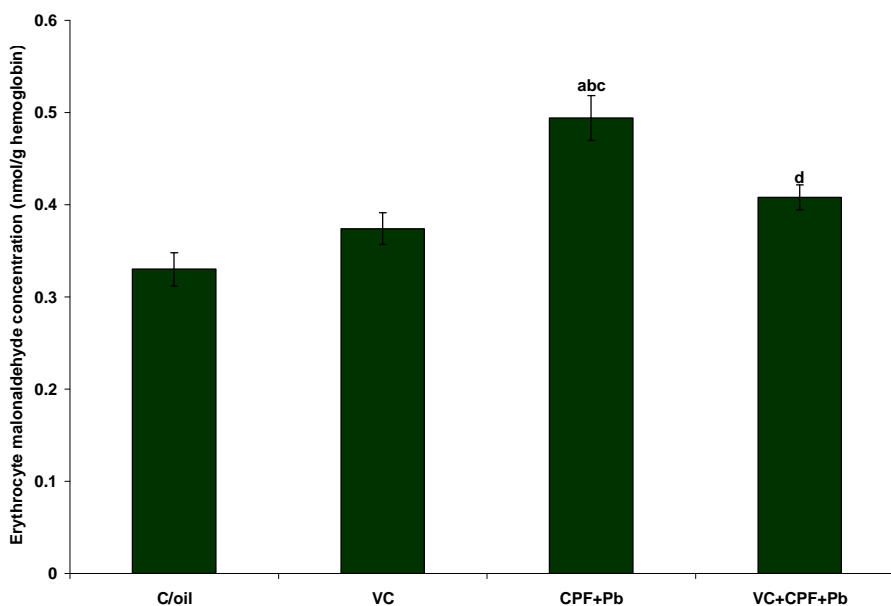


Figure 2: Effect of corn oil (C/oil), vitamin C (VC) and/or co-administration of chlorpyrifos (CPF) and lead (Pb) on erythrocyte malonaldehyde concentration in Wistar rats^{abc} $P < 0.01$ versus C/oil, VC and VC+CPF+Pb, respectively; ^d $P < 0.05$ versus C/oil

Table 2: Effect of chronic exposure to corn oil (C/oil), vitamin C (VC) and/or chlorpyrifos (CPF) on absolute total and differential leukocyte count in Wistar rats.

Parameters	C/oil	VC	CPF+Pb	VC+CPF+Pb
Total leukocyte count ($\times 10^9/L$)	6.0 ± 0.5	6.0 ± 0.78	5.3 ± 0.18	5.7 ± 0.9
Neutrophils count ($\times 10^9/L$)	1.79 ± 0.18	3.0 ± 0.35	3.4 ± 0.57	3.6 ± 0.6
Lymphocytes count ($\times 10^9/L$)	4.1 ± 0.6	2.4 ± 0.6	1.520 ± 0.13^a	1.8 ± 0.12^a
Monocytes count ($\times 10^9/L$)	0.0 ± 0.0	0.3 ± 0.3	0.3 ± 0.3	0.0 ± 0.0^f
Eosinophil count ($\times 10^9/L$)	0.0 ± 0.0	0.3 ± 0.3	0.3 ± 0.3	0.0 ± 0.0
Neutrophil:Lymphocyte ratio	0.4 ± 0.14	1.5 ± 0.5	2.2 ± 0.39	2.1 ± 0.5

^a $P < 0.05$ versus C/oil

DISCUSSION

The lower PCV and concentrations of Hb and RBC in the CPF+Pb group revealed the presence of anemia in this group. Krishna and Ramachandran [17] showed that co-administration of Pb and Pb in rats causes anemia. Similarly, earlier studies have shown anemia in CPF [13, 18, 19, 20] and Pb [21, 22] poisoning. The reason for the anemia in CPF poisoning has been associated with its ability to decrease serum iron concentration [19] thereby interfering with hemoglobin synthesis. Similarly, increased erythrocyte MDA concentration indicating membrane lipoperoxidation which was recorded in the present study indicate an increased oxidative damage to the erythrocyte membranes, which has been associated with increased RBC fragility [8, 20, 23]. In addition, renal lesions, which has been associated with CPF [13] and other OP poisonings may have contributed to the anemic condition in the group co-administered CPF and lead, apparently due to erythropoietin deficiency [24]. Lead induces anemia by interfering with heme biosynthesis through inhibition of δ -aminolevulinic acid dehydratase and ferrochelatase activities,

and by also decreasing erythrocytes survival [22, 25]. In addition, the interference of lead with the development of hematopoietic progenitor and alteration of production of renal erythropoietin are increasingly being linked with anemia in lead poisoning [26]. Furthermore, the anemia may be as a result of lead interference with copper metabolism [21]. The comparatively high MCV and low MCHC shows that the anemia recorded following co-administration of CPF and Pb was apparently of macrocytic hypochromic type. This finding contradicted the normocytic hyperchromic anemia reported in CPF [20] and microcytic hypochromic anemia in lead poisoning [21]. Anisocytosis recorded in the group co-administered CPF and Pb is a characteristic feature of moderate to severe anemia. Anisocytosis had been previously reported in CPF [19, 20] and lead [27, 28] poisonings.

Vitamin C was able to mitigate the anemia induced by co-exposure to CPF and lead. The mitigation of the anemia may be due to the ability of the vitamin to improve iron absorption [29] and reduce lipoperoxidative damage to the erythrocyte membranes, which has been demonstrated in the present and previous studies [8].

The present study also revealed that subchronic co-exposure to CPF and Pb resulted in relative leucopenia apparently due to lymphopenia. Chronic CPF exposure had been shown to cause lymphopenic leucopenia [13, 19, 20]. Similarly, repeated lead exposure has been demonstrated to induce leucopenia [30]. The apparent increase in NLR in the CPF + Pb group, which also indicates neutrophilia, shows the level of stress being experienced by the rats in this group. The leukopenia induced in the CPF+Pb group may have resulted from oxidative damage to the leukocytes. Pretreatment with vitamin C has been shown by the present study to apparently improve the leukocyte count and reduced the NLR. This shows that the vitamin protected the leukocytes from destruction apparently due to its antioxidant properties. The reduction in the NLR in the VC+CPF+Pb group suggests that the vitamin reduces the level of stress imposed on the animal by the co-administered CPF and lead.

The thrombocytopenia recorded in the CPF+Pb group may have been due to oxidative damage to the platelet membranes. This results in the formation of lipid peroxides within the platelet membranes thereby provoking cellular lysis. The platelet membrane is highly vulnerable to oxidative stress than the erythrocyte membrane [31]. The improvement in platelet count in the group pretreated with vitamin C shows the ability of the vitamin to protect the platelet from oxidative damage by reducing the formation of lipid peroxides within the platelet membranes. This results in the improvement of cellular integrity and reduction of cellular destruction.

The increase in the erythrocyte MDA concentration in the CPF+Pb group indicates increased lipoperoxidation. Chronic CPF exposure has been shown in our earlier studies to induce increased erythrocyte lipoperoxidation [8, 20, 23]. This increased lipoperoxidative changes compromises the structural integrity of the erythrocytes thereby resulting in increased vulnerability to lysis. This may have contributed to the anemia recorded in the CPF+Pb group. Pretreatment with vitamin C was able to mitigate the erythrocyte lipoperoxidative changes thereby strengthening the membrane integrity of the RBC hence ameliorating the anemia evoked by co-administration of CPF and Pb. This protective effect of vitamin C may be partly due to its antioxidant properties.

Apart from its antioxidant properties, the protective effect of vitamin C on hemotoxicity induced by co-administration of CPF and Pb in the present study may be partly due to its non-antioxidant properties. Vitamin C has been shown to increase the activity of PON I [32], an esterase enzyme that aid in the detoxification of OP compounds. Similarly, vitamin C also chelates lead [33], thereby reducing the concentration that directly interacts with the tissue.

In conclusion, the present study has shown that co-exposure to CPF and Pb results in alterations of hematological parameters partly due to induction of cellular lipoperoxidation. Similarly, vitamin C has been shown by the present study to mitigate the hemotoxicity induced by co-exposure to CPF and Pb partly due to its antioxidant properties.

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