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Chlorpyrifos-Induced Alteration of Hematological Parameters in Wistar Rats: Ameliorative Effect of Zinc

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Abstract: Chlorpyrifos (CPF) toxicity has been shown to be partly mediated through induction of oxidative stress. In the present study, experiments were conducted with the aim of evaluating the ameliorative potentials of zinc on CPF-evoked alteration in hematological parameters in Wistar rats. Twenty adult male Wistar rats were divided at random into 4 groups of 5 animals per group. Rats in group I served as the control and were given only soya oil (2 mL kg⁻¹) while those in group II were administered zinc only (50 mg kg⁻¹). Rats in group III were dosed with CPF only (10.6 mg kg⁻¹, ~1/8th of LD₅₀). Those in group IV were co-administered zinc (50 mg kg⁻¹) and CPF (10.6 mg kg⁻¹). The different regimens were administered orally once daily for a period of 8 weeks. At the end of the study period, blood samples collected after sacrificing the animals were analyzed for hematologic parameters such as Packed Cell Volume (PCV), Hemoglobin (Hb), Red Blood Cells (RBC), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), hemoglobin indices, platelets, absolute and differential White Blood Cell (WBC) counts, neutrophil-lymphocyte ratio and erythrocyte malonaldehyde (MDA) concentration. The results revealed that alterations in the values of PCV, Hb, RBC, platelets, absolute and differential WBC and MDA induced by CPF were ameliorated by co-administration with zinc. In conclusion, the study has shown that attenuations of CPF-evoked alterations in hematological parameters by zinc may be partly mediated through its antioxidant properties.

Key words: Organophosphate insecticides, chlorpyrifos, hematology, lipoperoxidation, amelioration, zinc

INTRODUCTION

Chlorpyrifos, a phosphorothioate broad spectrum moderately toxic Organophosphate (OP) insecticide remains one of the most widely used in agriculture and public health, despite the restriction of some of its domestic uses by the United States Environmental Protection Agency in 2000. The primary mechanism of toxicity is associated with its ability and especially that of its metabolite, CPF-oxon to inhibit acetylcholinesterase (AChE), an enzyme that normally terminates neurotransmission at cholinergic synapses (Eaton *et al.*, 2008). However, other mechanisms independent of AChE inhibition have been reported since, toxicity occurs at doses that did not inhibit the enzyme (Slotkin, 2004, 2005; Slotkin *et al.*,

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2007). Therefore, rather operating through a single defined mechanism, CPF toxicity involves several families of mechanisms. Among these, the induction of oxidative stress has been given considerable attention (Gultekin *et al.*, 2001; Ambali *et al.*, 2007; Tuzmen *et al.*, 2008). Prolonged exposure to CPF has been shown to cause anemia (Ambali, 2009). Although, the mechanism of CPF-induced anemia has not been elucidated, earlier study from our laboratory suggests that increased erythrocyte fragility apparently due to oxidative damage to the erythrocyte membranes may be partly involved (Ambali *et al.*, 2009).

Zinc is the second most abundant trace element in the body. It is particularly important in cellular function because it is an integral component of numerous proteins, including metalloenzymes, structural proteins and transcriptional factors (Zhou *et al.*, 2007). The antioxidant effect of zinc have been clearly demonstrated in several studies (Korbashi *et al.*, 1989; Goel *et al.*, 2005; Brocardo *et al.*, 2007). Zinc has never been shown to interact directly with oxidant species but rather prefers to exert its effects in an indirect manner (Powell, 2000). Adequate zinc intake is an important determinant of the antioxidant capacity (Koury *et al.*, 2004; Kamp and Donangelo, 2008; Oliveira *et al.*, 2009). The present study investigated the ameliorative effect of zinc on CPF-evoked hematological alteration in Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Twenty male Wistar rats (12-14 weeks old) weighing 115-126 g used for this study were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria. They were fed on pellets made from grower's mash, maize bran and groundnut cake in the ratio of 4:2:1 and water was provided *ad libitum*. The study was conducted in the Toxicology Laboratory of the Department.

Chemicals

Commercial grade CPF, Termicot® (Sabero Organics, Gujarat Limited, India) used for this study was reconstituted in soya oil (1%) prior to daily administration. Zinc gluconate tablet (50 mg; Nature Field, USA) was obtained from a Pharmaceutical store in Zaria, Nigeria. They were reconstituted in distilled water (50 mg mL⁻¹) prior to daily administration.

Experimental Protocol

The rats were weighed and then divided at random into 4 groups with each group having 5 animals. Rats in group I served as the control (labeled S/oil) and were given only soya oil (2 mL kg⁻¹). Rats in group II (labeled Zn) were administered zinc only (50 mg kg⁻¹) while those in group III (labeled CPF) were dosed with CPF only at 10.6 mg kg⁻¹, ~1/8th of LD₅₀ (Ambali, 2009). Group IV (labeled Zn+CPF) were co-administered with zinc (50 mg kg⁻¹) and CPF (10.6 mg kg⁻¹). The different regimens were administered by oral gavage once daily for a period of 8 weeks. At the end of the study period, the rats were sacrificed by severing the jugular vein after light ether anesthesia. The study was carried out according to the specifications of the Ahmadu Bello University Animal Research Committee and in accordance with Helsinki Declaration.

Hematological Evaluation

Two milliliter of blood collected into heparinized sample bottles were analyzed for 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 (Gulye *et al.*, 1988), while the neutrophil-lymphocyte ratio was calculated. The hemoglobin index was obtained using the formular described by Smirnov *et al.* (1987).

Evaluation of Erythrocyte Lipid Peroxidation

Three milliliter of heparinized blood sample obtained from each animal was centrifuged at 1000x g and the plasma discarded. Erythrocyte packets were prepared by washing erythrocytes three times in cold isotonic saline (0.9% w/v). The washed erythrocytes were used to analyze for malondialdehyde (MDA) concentrations using the double heating method of Draper and Hadley (1990) as modified by Altuntas *et al.* (2002). The principle of the method was based on spectrophotometric measurement of the colour produced during the reaction of thiobarbituric acid (TBA) with MDA. Briefly, 2.5 mL of 100 g L⁻¹ trichloroacetic acid solution was added to 0.5 mL of erythrocytes in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1000x g for 10 min and 2 mL of the supernatant was added to 1 mL of 6.7 g L⁻¹ TBA in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance measured using a UV spectrophotometer (Jenway, 6405 model, Japan) at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex, 1.56×10⁵/cm/M and expressed in nmol g⁻¹ of hemoglobin. The hemoglobin concentration was determined using the method described by Dacie and Lewis (1991).

Statistical Analysis

Values obtained as Mean±SEM were subjected to one-way Analysis of Variance (ANOVA) followed by Tukey test using GraphPad Prism version 4.0 for windows from GraphPad Software, San Diego, California, USA (www.graphpad.com). Values of p<0.05 were considered significant.

RESULTS

Effects of Treatments on Packed Cell Volume

The PCV of rats in the CPF group was significantly lower (p<0.05) compared to either the S/oil, Zn or Zn+CPF group. There was no significant change in the PCV of rats in the Zn+CPF group compared to either the S/oil or Zn group (Fig. 1).

Effect on Hemoglobin Concentration

The Hb concentration in the CPF group was significantly lower (p<0.05) compared to either the S/oil, Zn or Zn+CPF group. There was no significant difference (p>0.05) in the PCV of Zn+CPF group compared to the S/oil and Zn groups (Fig. 2)

Effect of Treatments on Total Red Blood Cell Concentration

The effect of treatments on RBC concentration is shown in Fig. 3. The RBC concentration in the CPF group was significantly lower (p<0.05) compared either the S/oil or Zn group. Anisocytosis was also observed in the CPF group compared to the other groups. The RBC concentration in the Zn+CPF group was marginally higher than those in the CPF group but it was not significant (p>0.05).

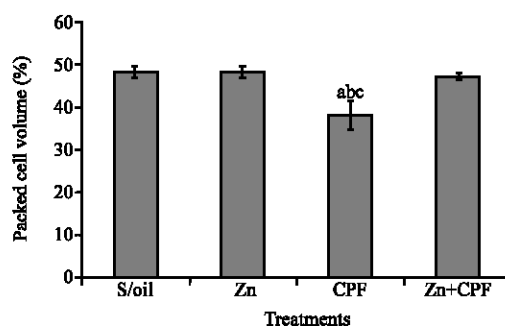


Fig. 1: Effect of soya oil, zinc and chlorpyrifos on packed cell volume. ^a $p < 0.01$ versus soya oil group; ^b $p < 0.05$ versus zinc group; ^c $p < 0.05$ versus zinc+chlorpyrifos group. Values are Mean $\pm$ SEM of 5 animals per group

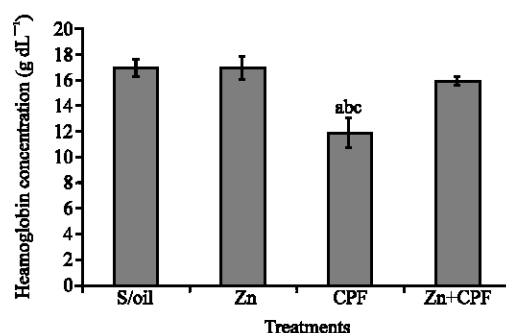


Fig. 2: Effect of soya oil, zinc and chlorpyrifos on hemoglobin concentration in Wistar rats. ^a $p < 0.05$ versus soya oil group; ^b $p < 0.01$ versus zinc group; ^c $p < 0.05$ versus zinc+chlorpyrifos group. Values are Mean $\pm$ SEM of 5 animals per group

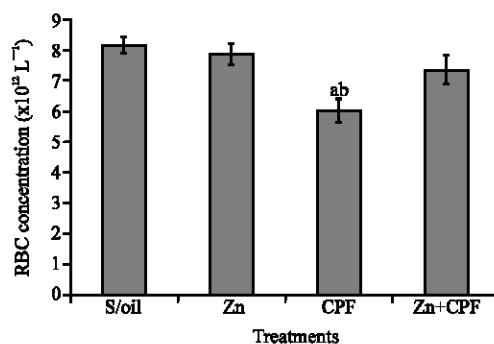


Fig. 3: Effect of soya oil, zinc and chlorpyrifos on red blood cell concentration in Wistar rats. ^a $p < 0.05$ versus soya oil group; ^b $p < 0.05$ versus zinc group. Values are Mean $\pm$ SEM of 5 animals per group

Effect of Treatments on Red Blood Cell Indices

The values obtained for MCV, MCH and MCHC were not significantly different ($p > 0.05$) between the groups (Fig. 4)

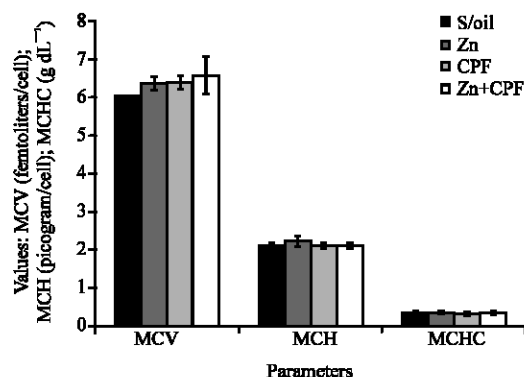


Fig. 4: Effect of soya oil, zinc and chlorpyrifos on red blood cell indices in Wistar rats. Values are Mean $\pm$ SEM of 5 animals per group

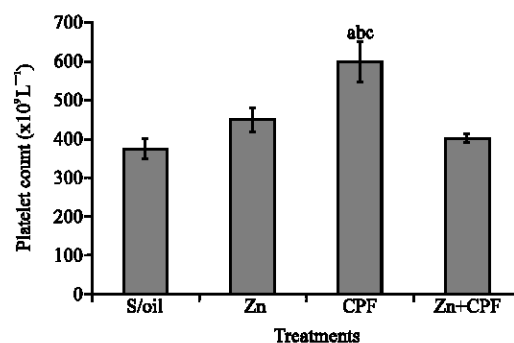


Fig. 5: Effect of soya oil, zinc and chlorpyrifos on platelet count in Wistar rats. ^ap<0.01 versus soya oil group; ^bp<0.05 versus zinc group; ^cp<0.01 versus zinc+chlorpyrifos group. Values are Mean $\pm$ SEM of 5 animals per group

Effect of Treatments on Platelet Count

The platelet count in the CPF group was significantly elevated compared to either the S/oil (p<0.01), Zn (p<0.05) or Zn+CPF (p<0.01) group. The platelet count in the Zn+CPF group did not significantly vary (p>0.05) from those obtained in the S/oil and Zn groups (Fig. 5).

Effect of Treatments on White Blood Cells

The WBC count in the CPF group was significantly lower (p<0.01) compared to either the S/oil, Zn or Zn+CPF group. There were no significant variation (p>0.05) in the WBC count in S/oil group compared to either Zn or Zn+CPF group (Fig. 6).

Effect of Treatments on Differential Leukocyte Count

The effect of treatments on differential leukocyte count is shown in Fig. 7. There was no significant difference in the neutrophil count between the groups. On the other hand, the lymphocyte count in the CPF group was significantly lower (p<0.01) compared to either the S/oil, Zn or Zn+CPF group. No significant change (p>0.05) in the lymphocyte concentration was recorded in the Zn+CPF group compared to either the S/oil or Zn group.

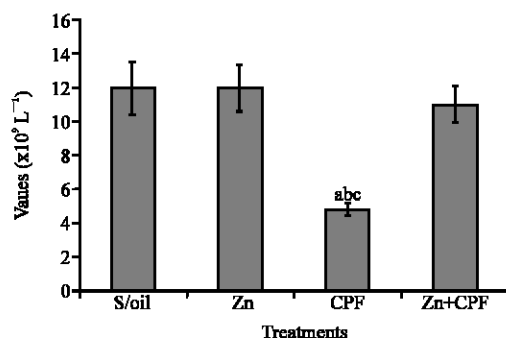


Fig. 6: Effect of soya oil, zinc and chlorpyrifos on total white blood cell count in Wistar rats. ^ap<0.05 compared to soya oil group. ^bp<0.01 versus soya oil; ^cp<0.05 versus zinc; ^dp<0.05 versus zinc+chlorpyrifos group. Values are Mean±SEM of 5 animals per group

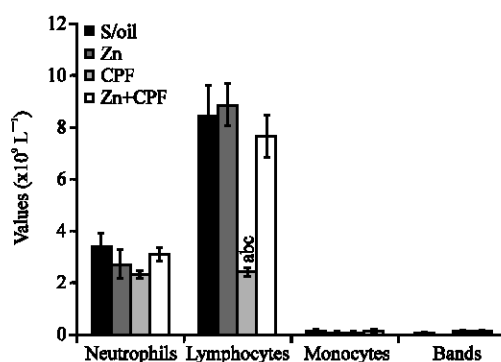


Fig. 7: Effect of soya oil, zinc and chlorpyrifos on differential white blood cell count in Wistar rats. ^ap<0.01 versus soya oil group. ^bp<0.01 versus soya oil group; ^cp<0.05 versus zinc group; ^dp<0.01 versus zinc+chlorpyrifos group. Values are Mean±SEM of 5 animals per group

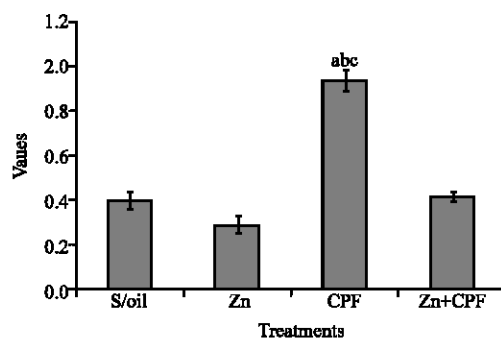


Fig. 8: Effect of soya oil, zinc and chlorpyrifos on lymphocyte/neutrophil ratio in Wistar rats. ^ap<0.01 versus soya oil group. ^bp<0.01 versus soya oil group; ^cp<0.05 versus zinc group; ^dp<0.01 versus zinc+chlorpyrifos group. Values are Mean±SEM of 5 animals per group

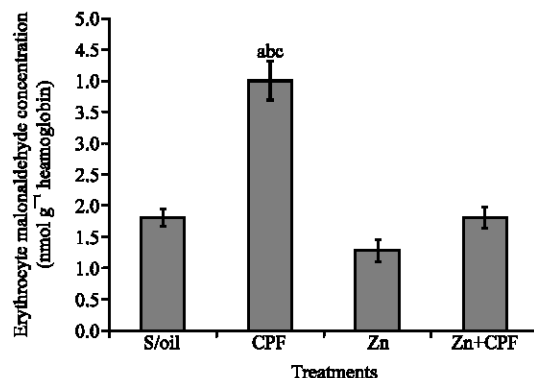


Fig. 9: Effect of soya oil, zinc and chlorpyrifos on erythrocyte malonaldehyde concentrations. ^aversus soya oil group ($p < 0.01$). ^bversus zinc group ($p < 0.01$); ^a $p < 0.01$ versus soya oil; ^b $p < 0.05$ versus zinc; ^c $p < 0.05$ versus zinc+chlorpyrifos group. Values are Mean $\pm$ SEM of 5 animals per group

Effect of Treatments on Neutrophil/Lymphocyte Ratio

The neutrophil/lymphocyte ratio in the CPF group was significantly higher ($p < 0.01$) compared to either the S/oil, Zn or Zn+CPF group. The neutrophil/lymphocyte ratio of Zn+CPF group was not significantly different ($p > 0.05$) from those obtained in the S/oil and Zn groups (Fig. 8).

Effect of Treatments on Erythrocyte Lipoperoxidative Changes

There was a significant elevation ($p < 0.01$) in the erythrocyte MDA concentrations in the CPF group compared to either the S/oil, Zn or Zn+CPF group. The MDA concentration in the Zn+CPF was not significantly different ($p > 0.05$) from those obtained in the S/oil and Zn+CPF groups (Fig. 9).

DISCUSSION

The lower values of PCV, Hb and RBC observed in the CPF group in the present study agreed with those obtained in earlier studies (Barna-Lloyd *et al.*, 1991; Goel *et al.*, 2006; Ambali, 2009). This indicates that repeated CPF exposure causes anemia. The lack of significant change in the MCV in all the groups shows that CPF does not alter erythrocyte size. However, the anisocytosis in the CPF group agreed with the observation of Goel *et al.* (2006). Anisocytosis is a feature that is commonly found in anemic and other blood conditions. The hemoglobin index of CPF group was greater than 1 (data not shown), indicating hyperchromicity (Smirnov *et al.*, 1987). The reason for the anemia in the CPF group is not known. It may however be related to disruption of erythropoiesis or an increase in RBC destruction (Vural *et al.*, 1986). Goel *et al.* (2006) observed a decrease in serum iron concentration which eventually results in reduced hemoglobin production. An earlier report from our laboratory has shown that repeated CPF exposure increases erythrocyte fragility due to increased lipoperoxidative changes in the erythrocyte membranes (Ambali *et al.*, 2009). The increased MDA concentration in the erythrocytes of rats exposed to CPF only is indicative of lipoperoxidative damage, which agreed with a previous report from our laboratory (Ambali *et al.*, 2009) and those recorded by other workers (Gultekin *et al.*, 2001;

Mansour and Mossa, 2009). Lipid peroxidation is a process that is determined by the extent of peroxide-deforming free radical mechanism on the Polyunsaturated Fatty Acids (PUFA) (Tuzmen *et al.*, 2008). The MDA is considered to be the most significant indicator of membrane lipid peroxidation resulting from the interaction between Reactive Oxygen Species (ROS) and cell membranes (Halliwell and Gutteridge, 1990, 1995). The RBC is vulnerable to lipoperoxidative changes because of its direct association with molecular oxygen, high content of metal ions catalyzing oxidative reactions and availability of high amount of PUFA, which are susceptible to lipid peroxidation. Inability to repair membrane damage and regenerate and poor antioxidant enzymes composition of the plasma medium in which they are bathed are some of the other factors that enhance the vulnerability of the RBC to lipid peroxidation (Marklund *et al.*, 1982; Etlik and Tomur, 2006). By-products of lipid peroxidation causes profound alterations in the structural organization and functions of the cell membrane including decreased membrane fluidity, increased membrane permeability, inactivation of membrane-bound enzymes and loss of essential fatty acids (Ginkel and Sevanian, 1994). CPF is lipophilic and may enhance lipid peroxidation by directly interacting with the cellular plasma membrane (Hazarika *et al.*, 2003). The overall effect of these on the RBC is the reduction in its membrane integrity and life span. Apart from the direct effect of oxidative stress, we have earlier demonstrated the ability of CPF to cause degenerative changes in the kidneys (Ambali, 2009), which may alter the production of erythropoietin, an hormone critical to functional erythropoiesis. Furthermore, CPF has been shown to cause chromosomal abnormality in the bone marrow (Anonymous, 2008). Therefore, the anemia observed following repeated CPF exposure is due to a combination of factors.

The significant improvement in the PCV, Hb and RBC concentrations and anisocytosis in rats co-administered zinc and CPF suggests the ameliorative effect of the trace element. The ameliorative effect of zinc on the indices of anemia agreed with the observation of Goel *et al.* (2006). The positive association between the improvement in anemia indices and amelioration of the erythrocyte lipoperoxidative changes demonstrated that oxidative damage probably plays an important role in CPF-induced anemia. The ameliorative effect of zinc on CPF-induced anemia may have arisen from its antioxidant properties (Powell, 2000). Cellular zinc exists in only one redox state (II); thus, it cannot undergo redox reactions that are commonly responsible for the generation of reactive oxygen species (Zhou *et al.*, 2007). Apart from its direct antioxidant effect via occupation of iron and copper binding sites on lipids, proteins and DNA thereby preventing hydroxyl radical formation near these structures (Powell, 2000; Prasad and Kucuk, 2002), zinc also plays a structural role in the maintenance of the integrity of Cu-Zn superoxide dismutase as a cofactor (Coudray *et al.*, 1992; Sahin and Kucuk, 2003) and in the regulation of glutathione that is vital to cellular antioxidant defense (Parrat *et al.*, 1997). In addition, zinc protects sulphhydryl group against oxidation thereby preventing protein from oxidation, hence stabilizing the cellular thiol pools (Kraus *et al.*, 1997; Verhaegh *et al.*, 1998). Furthermore, zinc regulates metallothionein, a highly conserved low molecular weight cystein rich metal chelating antioxidant protein, which has been demonstrated to share a lot of features with glutathione (Quesada *et al.*, 1996; Zhou *et al.*, 2007). Goel *et al.* (2005) have shown that CPF intoxication causes reduction in metallothionein level, a feature that was ascribed to zinc deficiency. Zinc deficiency has been associated with increased erythrocyte fragility (Kraus *et al.*, 1997; O'Dell, 2000) apparently due to the alteration in erythrocyte membrane integrity. A decrease in membrane zinc content could cause subtle changes in membrane rheology and leave vacant potential binding sites for redox-active metals such as iron and copper (Driscoll and Bettger, 1992) that can promote membrane-lipid oxidation (Verstraeten *et al.*, 2004) and alterations in membrane lipid

composition (Driscoll and Bettger, 1992). This leads to disturbance in the physical properties of the lipid bilayers (Verstraeten *et al.*, 2004). Although, the present study did not measure the level of zinc in the erythrocyte membrane, it is no gain saying that CPF-induced zinc deficiency as observed in a previous study (Goel *et al.*, 2005) may have played a significant role in the pathophysiology of anemia observed in the present study. Therefore, zinc supplementation may have attenuated CPF-induced zinc deficiency and hence ameliorates the anemia. Zinc supplementation has been shown to cause positive haematological parameters in athletes (Kilic *et al.*, 2004).

The study also recorded a significant increase in platelet count of rats exposed to CPF only. This agreed with the findings of Szabo *et al.* (1988). The implication of thrombocytosis in rats exposed to low-dose CPF is not known. However, thrombocytosis is associated with coagulopathic disorders characterized by widespread clotting in the tissue, resulting in embolism, tissue ischemia and tissue/organ damage. ROS has been shown to facilitate platelet activation (Krotz *et al.*, 2004; Essex, 2009). Zinc supplementation has been shown by the present study to normalize the CPF-evoked thrombocytosis. Although, the mechanism responsible for this is not clear, it may be partly due to the antioxidant effect of zinc resulting in lowered ROS elaboration.

Repeated CPF exposure has been shown by the present study to cause lymphopenic leukopenia. This finding agreed with that obtained in our earlier studies (Ambali *et al.*, 2007; Ambali, 2009). The lymphopenia observed in the CPF-exposed rats may be due to either decreased production and/or increased rate of removal due to rapid destruction. Although, the present study did not observe significant change in the neutrophil content of the blood, Goel *et al.* (2006) observed neutrophilia following CPF exposure. The high neutrophil: lymphocyte ratio in the CPF group in the present study however corroborated high neutrophil count observed in the earlier study (Goel *et al.*, 2006) indicating that the animals exposed to this chemical are under stress. Earlier works have shown that pesticides are toxic to the cells of the immune system through the induction of necrosis and apoptosis. Immune cells are particularly sensitive to oxidative stress due to the high percentage of PUFA in their plasma membranes and the increased production of ROS (Knight, 2000). Oxidative stress provoked by CPF may have played a significant role in cellular necrosis and apoptosis. Supplementation with zinc has been shown in the present study to attenuate CPF-evoked leukopenia and increased neutrophil:lymphocyte ratio. This demonstrated that zinc supplementation improved the CPF-induced lymphopenia and stress. Zinc plays an essential role in the T-cell function. Zinc ions have been found to induce blast transformation in human lymphocytes (Rühl *et al.*, 1971; Berger and Skinner, 1974). Zinc is therefore unique as the simplest mitogen known (Wellinghausen and Rink, 1998). Similarly, zinc is an essential cofactor for thymulin, an important thymic hormone secreted by thymic epithelial cells (Hadden, 1992). Apart from inducing markers of differentiation in immature T cells in the thymus, thymulin also has effects in the periphery where it influences mature T cells (Wellinghausen and Rink, 1998). Furthermore, the protective effect of zinc on WBC may be attributed to their antioxidant properties either as a vital component of enzymatic antioxidant Cu-Zn SOD or due to their ability to antagonize the catalytic properties of the redox-active transition metals iron and copper with respect to the promotion of hydroxyl radicals formation from hydrogen peroxide and superoxide (Powell, 2000). Zn also causes inhibition of both endogenous as well as induced lipid peroxidation thereby resulting in stabilization of biomembranes (Dhawan *et al.*, 1992; Srivastava *et al.*, 1993).

In conclusion, the present study has shown that zinc supplementation attenuated CPF-induced alteration in hematological parameters partly due to its antioxidant activity. Therefore, individuals who are constantly exposed to low-dose of CPF and perhaps other OP insecticides may benefit from toxicity protection offered by zinc supplementation.

REFERENCES

- Altuntas, I., N. Delibas and R. Sutcu, 2002. The effects of organophosphate insecticide methidathion on lipid peroxidation and anti-oxidant enzymes in rat erythrocytes: Role of vitamins E and C. *Hum. Exp. Toxicol.*, 21: 681-685.
- Ambali, S., D. Akanbi, N. Igbokwe, M. Shittu, M. Kawu and J. Ayo, 2007. Evaluation of subchronic chlorpyrifos poisoning on hematological and serum biochemical changes in mice and protective effect of vitamin C. *J. Toxicol. Sci.*, 32: 111-120.
- Ambali, S.F., 2009. Ameliorative effect of vitamins C and E on neurotoxicological, hematological and biochemical changes induced by chronic chlorpyrifos in Wistar rats. Ph.D. Thesis, Ahmadu Bello University, Zaria, pp: 356.
- Ambali, S.F., J.O. Ayo, S.A. Ojo and K.A.N. Esievo, 2009. Ameliorative effect of vitamin C on chronic chlorpyrifos-induced erythrocyte osmotic fragility in Wistar rats. *Hum. Exp. Toxicol.*
- Anonymous, 2008. Chlorpyrifos/Dursban. <http://en.wikipedia.org/wiki/Chlorpyrifos>.
- Barna-Lloyd, T., J.R. Szabo and N.L. Davis, 1991. Chlorpyrifos-Methyl (Reldan R) Rat Chronic Dietary Toxicity/Oncogenicity Study. WHO, Indianapolis, USA.
- Berger, N.A. and M. Skinner, 1974. Characterization of lymphocyte transformation induced by zinc ions. *J. Cell. Biol.*, 61: 45-55.
- Brocardo, P.S., F. Assini, J.L. Franco, P. Pandolfo and M.R. Müller *et al.*, 2007. Zinc attenuates malathion-induced depressant-like behavior and confers neuroprotection in the rat brain. *Toxicol. Sci.*, 97: 140-148.
- Coudray C., M.J. Richard, F. Laporte, P. Faure, A.M. Roussel and A.E. Favier, 1992. Superoxide dismutase and zinc status: A study in man and animals. *J. Nutr. Med.*, 3: 13-26.
- Dacie, J.V. and S.M. Lewis, 1991. *Practical Haematology*. 7th Edn., Churchill Livingstone, London.
- Dhawan, D., A. Goel and C.S. Gautam, 1992. Effects of zinc intake on liver enzymes in carbontetrachloride induced liver injury. *Med. Sci. Res.*, 20: 55-56.
- Draper, H.H. and M. Hadley, 1990. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol.*, 186: 421-431.
- Driscoll, E.R. and W.J. Bettger, 1992. Zinc deficiency in the rat alters the lipid composition of the erythrocyte membrane Triton shell. *Lipids*, 27: 972-977.
- Eaton, D.L., R.B. Daroff, H. Autrup, J. Bridge and P. Buffler *et al.*, 2008. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. *Crit. Rev. Toxicol.*, 38: 1-125.
- Essex, D.W., 2009. Redox control of platelet function. *Antioxidants Redox Signaling*, 11: 1191-1225.
- Etlík, Ö. and A. Tomur, 2006. The oxidant effects of hyperbaric oxygenation and air pollution in erythrocyte membranes (hyperbaric oxygenation in air pollution). *Eur. J. Gen. Med.*, 3: 21-28.
- Ginkel, G.V. and A. Sevanian, 1994. Lipid peroxidation induced membrane structural alterations. *Method. Enzymol.*, 233: 273-288.
- Goel, A., V. Danni and D.K. Dhawan, 2005. Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. *Chem. Biol. Interact.*, 156: 131-140.
- Goel, A., V. Danni and D.K. Dhawan, 2006. Role of zinc in mitigating the toxic effects of chlorpyrifos on hematological alterations and electron microscopic observations in rat blood. *BioMetals*, 19: 483-492.

- Gultekin, F., N. Delibas, S. Yasar and I. Kilinc, 2001. *In vivo* changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. *Arch. Toxicol.*, 75: 88-96.
- Gulye, J.V., J.Z. Camicas and A.M. Diouf, 1988. Ticks and blood parasites in Senegal (*Sahlian zone*). *Rev. Elevage Med. Vet. Pays Trop.*, 40: 119-125.
- Hadden, J.W., 1992. Thymic endocrinology. *Int. J. Immunopharmacol.*, 14: 345-352.
- Halliwell, B. and J.M.C. Gutteridge, 1990. Role of free radicals and catalytic metal ions in human disease: An overview. *Method Enzymol.*, 186: 1-85.
- Halliwell, B. and J.M. Gutteridge, 1995. The definition and measurement of antioxidants in biological systems. *Free Radic. Biol. Med.*, 18: 125-126.
- Hazarika, A., S.N. Sarkar, S. Hajare, M. Katarina and J.K. Malik, 2003. Influence of malathion pretreatment on the toxicity of anilofos in male rats: A biochemical interaction study. *Toxicology*, 185: 1-8.
- Kamp, F. and C. Donangelo, 2008. Supplementating young women with both zinc and iron protects zinc related antioxidant indicators previously impaired by iron supplementation. *J. Nutr.*, 138: 2186-2189.
- Kilic, M., A.K. Baltaci and M. Gunay, 2004. Effect of zinc supplementation on hematological parameters in athletes. *Biol. Trace Element Res.*, 100: 31-38.
- Knight, J.A., 2000. Review: Free radical, antioxidants and the immune system. *Ann. Clin. Lab. Sci.*, 30: 145-158.
- Korbashi, P., J. Katzhandler, P. Saltman and M. Chevion, 1989. Zinc protects *E. coli* against copper-mediated paraquat-induced damage. *J. Biol. Chem.*, 264: 8479-8482.
- Koury, J.C., A.V. Oliveira, E.S. Portella, C.F. Oliveira, G.C. Lopes and C.M. Donangelo, 2004. Zinc and copper biochemical indices of antioxidant status in elite athletes of different modalities. *Int. J. Sport Nutr. Exerc. Metabol.*, 14: 358-372.
- Kraus, A., H. Roth and M. Kirchgessner, 1997. Supplementation with vitamin C, vitamin E or β -carotene influences osmotic fragility and oxidative damage of erythrocytes of zinc-deficient rats. *J. Nutr.*, 127: 1290-1296.
- Krotz, F., H.Y. Sohn and U. Pohl, 2004. Reactive oxygen species: Players in the platelet game. *Arterioscler. Thrombosis Vasc. Biol.*, 24: 1988-1996.
- Mansour, S.A. and A.H. Mossa, 2009. Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. *Pesticides Biochem. Physiol.*, 93: 34-39.
- Marklund, S.L., E. Holme and L. Hellner, 1982. Superoxide dismutase in extracellular fluids. *Clin. Chim. Acta*, 126: 41-51.
- O'Dell, B.L., 2000. Role of zinc in plasma membrane function. *J. Nutr.*, 130: 1432-1436.
- Oliveira, K.J.F., C.M. Donagelo, A.V. Oliveira, C.L.O. Silveira and J.C. Koury, 2009. Effect of zinc supplementation on the antioxidant, copper and iron status of physically active adolescents. *Cell Biochem. Funct.*, 27: 162-166.
- Parrat, M.O., M.J. Richard, J.C. Beani and A. Favier, 1997. Involvement of zinc in intracellular oxidant/antioxidant balance. *Biol. Trace Element Res.*, 60: 187-204.
- Powell, S.R., 2000. The antioxidant properties of zinc. *J. Nutr.*, 130: 1447-1454.
- Prasad, A.S. and O. Kucuk, 2002. Zinc in cancer prevention. *Cancer Metastasis Rev.*, 21: 291-295.
- Quesada, A.R., R.W. Byrnes, S.O. Krezoski and D.H. Pettering, 1996. Direct reaction of H_2O_2 with sulfhydryl groups in HL-60 cells: Zinc-metallothionein and other sites. *Arch. Biochem. Biophys.*, 334: 241-250.

- Rühl, H., H. Kirchner and G. Borchert, 1971. Kinetics of the Zn²⁺-stimulation of human peripheral lymphocytes *in vitro*. *Proc. Soc. Exp. Biol. Med.*, 137: 1089-1092.
- Sahin, K. and O. Kucuk, 2003. Zinc supplementation alleviates heat stress in laying Japanese quail. *J. Nutr.*, 133: 2808-2811.
- Slotkin, T.A., 2004. Cholinergic systems in brain development and disruption by neurotoxicants: Nicotine, environmental tobacco smoke, organophosphates. *Toxicol. Appl. Pharmacol.*, 198: 132-151.
- Slotkin, T.A., 2005. Developmental Neurotoxicity of Organophosphates: A Case Study of Chlorpyrifos. In: *Toxicity of Organophosphate and Carbamate Pesticides*, Gupta, R.C. (Eds). Elsevier Academic Press, San Diego, pp: 293-314.
- Slotkin, T.A., E.A. MacKillop, I.T. Ryde, C.A. Tate and F.J. Seidler, 2007. Screening for developmental neurotoxicity using PC12 cells: Comparisons of organophosphates with a carbamate, an organochlorine and divalent nickel. *Environ. Health Perspect.*, 115: 93-101.
- Smirnov, A.M., P.Y. Konopelko, S.P. Pushkov, V.S. Postnikov and N.A. Urazav *et al.*, 1987. Methods of Clinical Examination. In: *Internal Non-Infectitious Diseases of Animals*, Smirnov, A.M. (Ed.). Kolov Press, Moscow, pp: 11-21.
- Srivastava, R.C., S.K. Hasan, J. Gupta and S. Gupta, 1993. Protective role of metallothionein in nickel induced oxidative damage. *Biochem. Mol. Biol. Int.*, 30: 261-370.
- Szabo, J.R., J.T. Young and M. Granjean, 1988. Chlorpyrifos: 13-week dietary toxicity study in fisher 344 rats. Health and Environmental Sciences-Texas, Laboratory Study No. TXT: K-044793-071. Report Dated Dec. 28, 1988. Reviewed by PMRA.
- Tuzmen, N., N. Candan, E. Kaya and N. Demiryas, 2008. Biochemical effects of chlorpyrifos and deltamethrin on altered antioxidative defense mechanisms and lipid peroxidation in rat liver. *Cell Biochem. Funct.*, 26: 119-124.
- Verhaegh, G., M.O. Parat, M.J. Richard and P. Hainut, 1998. Modulation of p53 protein conformation and DNA binding activity by intracellular chelation of zinc. *Mol. Carcinog.*, 21: 205-214.
- Verstraeten, S.V., M.P. Zago, G.G. Mackenzie, C.L. Keen and P.I. Oteiza, 2004. Influence of zinc deficiency on cell-membrane fluidity in Jurkat, 3T3 and IMR-32 cells. *Biochem. J.*, 378: 579-587.
- Vural, S., E.T. Cetin and U. Tuzlaci, 1986. Klinik teshiste laboraturvar. Nutrettin Uycan Ciltve, Basim Sanayii, Istanbul. <http://www.nadirkita.com/klinik-teshistelaboratuvar-1986-kitap635537.html>.
- Wellinghausen, N. and L. Rink, 1998. The significance of zinc for leukocyte biology. *J. leukocyte Biol.*, 64: 571-577.
- Zhou, Z., X. Kang, Y. Jiang, Z. Song, W. Feng, C.J. McClain and Y.J. Kang, 2007. Preservation of hepatocyte nuclear factor-4á is associated with zinc protection against TNF-á hepatotoxicity in mice. *Exp. Biol. Med.*, 232: 622-628.