Ameliorative Effect of Zinc on Chlorpyrifos-Induced Erythrocyte Fragility in Wistar Rats

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Abstract: Oxidative stress has been implicated in the molecular mechanism of chlorpyrifos (CPF) poisoning. The present study was aimed at evaluating the ameliorative effect of zinc, an antioxidant trace element, on lipoperoxidative changes and erythrocyte fragility induced by subchronic CPF exposure in Wistar rats. Twenty adult Wistar rats divided into 4 groups of 5 animals each were used as experimental subjects. Rats in groups I (S/oil group) and II (CPF group) were exposed to soya oil only (2 ml/kg) and CPF only (10.6 mg/kg~ $1/8^{th}$ LD_{50}), respectively. Group III (Zn group) were dosed with zinc only (50 mg/kg) while in group IV (Zn+CPF), Zn (50 mg/kg) were co-administered with CPF (10.6 mg/kg). These regimens were administered orally once daily for a period of 8 weeks. At the end of the study, the rats were sacrificed and erythrocytes analysed for erythrocyte osmotic fragility and malonaldehyde (MDA) concentration as an index of lipoperoxidative changes. Erythrocyte fragility was significantly increased ($P < 0.01$) in CPF group compared to S/oil, Zn and Zn+CPF groups, respectively. Similarly, the CPF group showed a significant increase ($P < 0.01$) in MDA concentration compared to S/oil, Zn and Zn+CPF groups, respectively.The present study has shown that repeated exposure of rats to CPF increased erythrocyte fragility partly due to oxidative hemolysis resulting from increased lipoperoxidative changes. [New York Science Journal 2010;3(5):117-122]. (ISSN 1554 – 0200).

Keywords: Chlorpyrifos, erythrocyte fragility, lipoperoxidation, amelioration, zinc

1. Introduction

Chlorpyrifos (CPF), a phosphorothionate chlorinated organophosphate insecticides has widespread agricultural, domestic and public health applications. Despite its beneficial uses, it causes adverse health consequence in non-target species, including man and animals. Earlier studies have shown that repeated exposure to CPF causes anemia (Ambali, 2009; Ambali et al., 2010). The mechanism of CPF-induced anemia have not been completely elucidated. Although the main mechanism of CPF toxicity involves inhibition of acetylcholinesterase (AChE), studies have shown that other mechanisms are involved in CPF poisoning since toxicity still occurs at levels that do not inhibit AChE or long after restoration of its activity (Pope et al., 1992; Caňadas et al., 2005). Oxidative stress induction is one of the molecular mechanisms that have been implicated in CPF poisoning (Gultekin et al., 2001; Ambali et al., 2007; Mansour and Mossa, 2009).

Zinc is the second most abundant trace element in the body (Zhou et al., 2007). It is contained in hundreds of enzymes and in even more protein domains, participating in a number of cellular processes, including cellular proliferation, differentiation and apoptosis (Franco et al., 2009). It is ubiquitous in sub-cellular metabolism and is an essential components of catalytic site(s) of at least one enzyme in every enzyme classification (Coyle et al., 2002). Zinc plays an important role in the structure and function of biological membranes (Bettger and O'Dell, 1993). Decreased zinc concentration in the RBC membrane is associated with increased erythrocyte fragility (Roth and Kirchgessner, 1991) . The antioxidant effect of zinc has been well documented (Moustafa, 2004; Zhou et al., 2005) Apart from being an essential component of the antioxidant enzyme, superoxide dismutase, zinc also antagonizes the catalytic properties of the redox active transition metals iron and copper in promoting the formation OH° from hydrogen peroxide and superoxide in Fenton reactions (Powell, 2000). Zinc also induces the expression of cystein-rich antioxidant protein, metallothionein (Dhawan and Goel, 1995). Zinc has been shown to suppress iron-mediated, xanthine/xanthine oxidase-induced erythrocytes membrane peroxidation in a biochemical system (Girotti et al., 1985). Previous studies have demonstrated the protective effect of zinc on chlorpyrifos-induced hemotoxicity (Ambali et al., 2010) and lipoperoxidative changes (Mansour and Mossa, 2009). Therefore, the present study was aimed at evaluating the ameliorative effect of zinc on CPF-induced erythrocyte fragility in Wistar rats.

2. Materials and Methods

2.1 Experimental animals

Twenty male Wistar rats (12-14 weeks old) weighing 115-126g used for this study were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria. The animal were housed in metal cages and fed on standard rat pellets, with water provided *ad libitum.*

2.2 Chemicals

Commercial grade chlorpyrifos, Termicot® (Sabero Organics, Gujarat Limited, India), a 20% EC was obtained from an Agrochemical store in Zaria, Nigeria. It was reconstituted to 1% in soya oil prior to daily administration. Zinc gluconate tablet (50 mg/tablet, Nature Field, USA) was obtained from a Pharmaceutical store in Zaria, Nigeria. They were reconstuituted in distilled water prior to daily administration.

2.3 Experimental protocol

The rats were weighed and then divided at random into 4 groups with each group having 5 animals. Group I served as the control (labeled S/oil) and were given only soya oil at the dose of 2ml/kg. Group II (labeled Zn) were administered zinc (50 mg/kg), while group III (labeled as CPF) were dosed with CPF only (10.6 mg/kg, ~$1/8^{th}$ LD_{50}). Group IV (labeled Zn+CPF) were co-administered zinc (50 mg/kg) and CPF (10.6 mg/kg). The different regimens were administered orally once daily for a period of 17 weeks. At the end of the study period, the rats were sacrificed by severing the jugular vein after light ether anesthesia and blood samples collected into heparinized sample bottles for the analysis of erythrocyte osmotic fragility and MDA concentrations. The study was carried out according to the specification of the Ahmadu Bello University Animal Research Committee.

2.4 Evaluation of erythrocyte osmotic fragility

The erythrocyte osmotic fragility was evaluated using the method described by Faulkner and King (1970) as modified by Oyewale (1991). Briefly, freshly obtained heparinized blood samples from each rat was pipetted into the test tubes containing 0.0, 0.1, 0.3, 0.5, 0.7, 0.9 g/L of NaCl (pH 7.4) and then followed by careful mixing and incubation for 30 minutes at room temperature, 26-28˚C. The test tubes were then centrifuged at 2000 x g for 10 minutes using a centrifuge model IEC HN-SII (Damon/IEC Division, UK). The supernatant was transferred into a glass cuvette and the absorbance of the supernatant measured colorimetrically with Spectronic 20 (Bausch and Lomb, USA) at wavelength of 540 nm. The percent hemolysis for each sample was then calculated using the following formula:

$$\% \text{ hemolysis} = \frac{\text{Optical density of test solution}}{\text{Optical density of standard solution}} \times 100$$

2.5 Evaluation of erythrocyte malonaldehyde concentration

Erythrocyte malonaldehyde (MDA) concentration, as a marker of lipid peroxidation was determined by the double heating method of Draper and Hadley (1990) as modified by Altuntas et al., (2002). The principle of the method was spectrophotometric measurement of the colour produced during the reaction of thiobarbituric acid (TBA) with MDA. Briefly, 2.5 ml of 100 g/L trichloroacetic acid 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 1000 x g for 10 min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/L TBA solution 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, 1.56×10^5 cm^{-1} M^{-1}, and expressed in nanomoles per gram of hemoglobin. The hemoglobin concentration was determined using the method of Dacie and Lewis (1991).

2.6 Statistical Analysis

Values obtained as mean ± SEM were subjected to one-way analysis of variance (ANOVA) followed by Tukey's 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.

3. Results

3.1 Effect on *in vitro* erythrocyte osmotic fragility

Hemolysis was completed (100%) in the standard solvent (distilled water). Generally, there were no significant changes ($P > 0.05$) in the degree of erythrocyte fragility among the rats in the various groups at 0.1, and 0.3 g/L of NaCl. On the other hand, there was a significant increase ($P < 0.05$) in the degree of erythrocyte fragility in the CPF group when compared to those in S/oil, Zn and Zn +CPF groups, respectively, at 0.5, 0.7 and 0.9 g/L of NaCl (Figure 1).

3.2 Effect on erythrocyte lipoperoxidative changes

The erythrocyte MDA concentrations used as an index of lipid peroxidation was significantly increased ($P < 0.01$) in the CPF group when compared to those obtained in the S/oil, Zn and Zn+CPF groups, respectively (Figure 2).

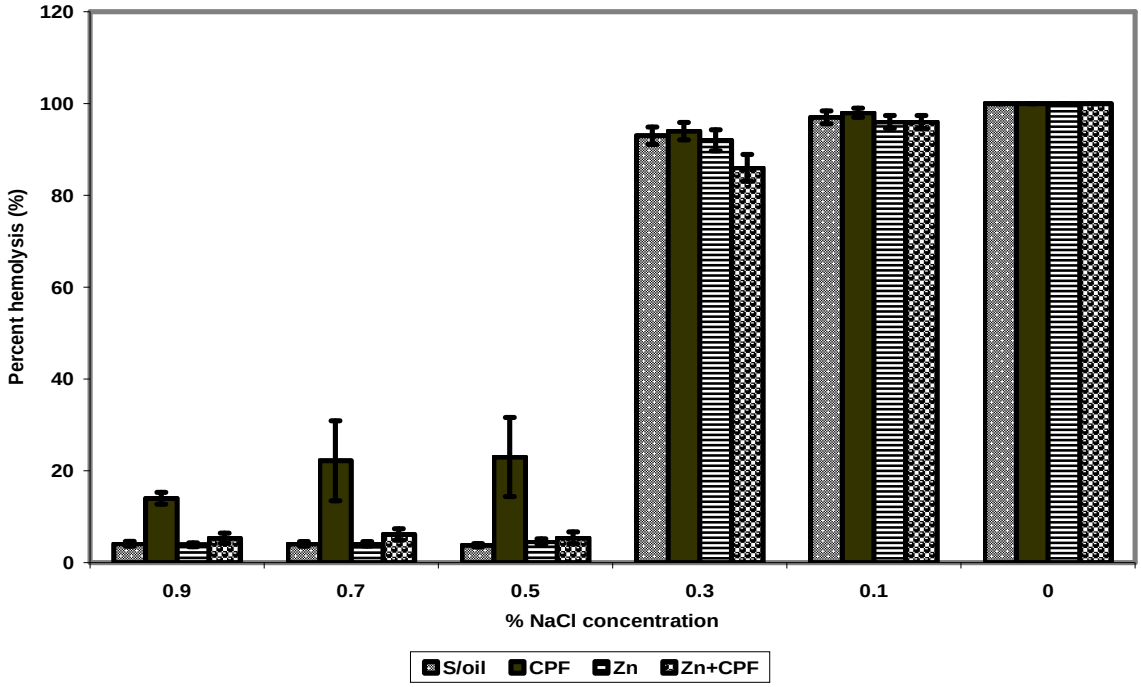

Figure 1: **Effect of soya oil, Zinc, chlorpyrifos, Zinc + chlorpyrifos on erythrocyte osmotic fragility.**

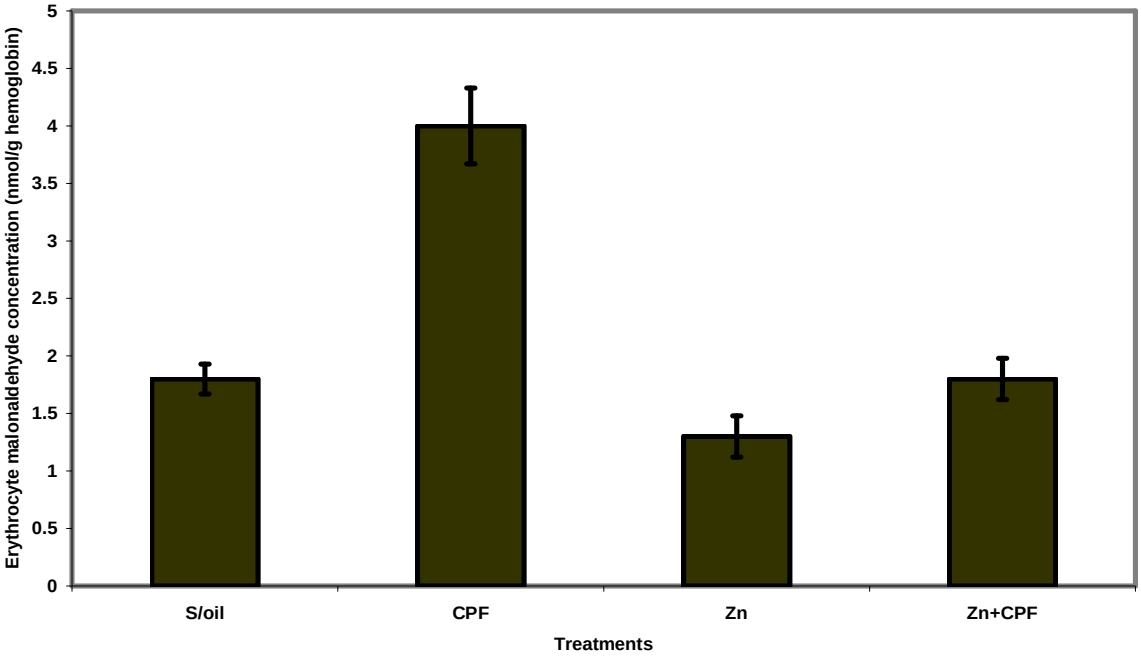

Figure 2: Effect of soya oil, Zinc, chlorpyrifos and Zinc + chlorpyrifos on erythrocyte malonaldehyde concentrations. [a]Comparison of chlorpyrifos group and soya oil group ($P < 0.01$). [b]Comparison of chlorpyrifos group and Zinc group ($P > 0.01$). [c]Comparison of chlorpyrifos group and Zinc + chlorpyrifos group ($P < 0.01$). Values are means ± SEM of 5 animals per group.

4. Discussion

The study showed a significant increase in the percentage erythrocyte fragility in the CPF group compared to the other group. This shows the ability of chronic OP insecticide exposure to compromise the integrity of the RBC membrane. The normal function of the RBC is largely hinged on the maintanance of the integrity of its membrane. The compromization of the RBC membrane integrity resulting in increased erythrocyte fragility in the group exposed to CPF only may have arisen from the increased lipoperoxidative changes as indicated by increased MDA concentration. This result agreed with those recorded in earlier studies that demonstrated increased lipoperoxidative changes and depletion of endogenous antioxidant enzymes in the erythrocytes of rats exposed to CPF (Gultekin et al., 2001; Mansour and Mossa, 2009). The oxidative modification of the erythrocyte membrane has been shown to increase the fragility of the RBC (Langsdorf and Zydney, 1993). Lipid peroxidation, which is the process of oxidative degradation of polyunsaturated fatty acids (PUFA) when it happens in biological membranes as shown in the CPF group causes impairment of membrane function and structural integrity (Gutteridge and Halliwell, 1988), decreased fluidity and inactivation of a number of membrane bound enzymes and protein receptors (Sidhu et al., 2004).

The constant exposure to high oxygen tension, high level of iron and richness in Polyunsaturated Fatty Acids (PUFA) (Kollanjiappan et al., 2002) coupled with their inability to possess nucleus and other organelles (Đorđević et al., 2008) have made erythrocyte a centre of free radical attack. Process of lipid peroxidation decreases hydrophobic characteristics of bilayer membrane of erythrocytes, altering affinity and interaction of proteins and lipids, thereby impairing the functioning and homeostasis of erythrocytes membrane (Dargel, 1991). Reactive Oxygen Species (ROS) can equally affect the proteins resulting in modification of enzymes activity and damage to the membrane transport proteins may produce disturbed cellular ionic homeostasis, leading to alterations in intracellular calcium and potassium that triggers a series of changes in the cell (Kerr et al., 1992). ROS can directly affect the conformation and/or activities of all sulfhydryl-containing molecules, by oxidation of their thiol moiety (Wilcox et al., 2001).

Co-administration of zinc has been demonstrated by the present study to significantly protect the erythrocytes from CPF-induced fragility and lipoperoxidative damage. A previous study has shown the ability of zinc to protect the erythrocyte from lipoperoxidative changes induced by CPF exposure (Mansour and Mossa, 2009). Therefore, the protective effects of zinc on CPF-induced lipoperoxidative changes and erythrocyte fragility may have arisen from its antioxidant properties. Cellular zinc exists in only one redox state (II); thus, it cannot undergo redox reactions that are commonly responsible for the generation of ROS (Zhou et al., 2007). Apart from its direct antoxidant effect by occupying iron and copper binding sites on lipids, proteins and DNA (Prasad and Kucuk, 2002), zinc also plays a structural role in the maintainance of the integrity of Cu-Zn superoxide dismutase as a cofactor (Sahin and Kucuk, 2003) and in regulation of glutathione that is vital to cellular antioxidant defense (Parrat et al., 1997). In addition, zinc protects sulphydryl group against oxidation thereby preventing protein from oxidation, hence stabilizing the cellular thiol pools (Kraus et al., 1997) may have been partly responsible for the stabilization of the RBC membrane in group preretreated with zinc.

Furthermore, zinc induces the production of metallothionein, an effective scavenger of hydroxyl radicals (Sahin and Kucuk, 2003). Therefore, the combined antioxidant activities of zinc may have been responsible for the decrease in lipoperoxidative changes and protective effect in CPF-induced erythrocyte fragility observed in this study. Besides, increase in erythrocyte fragility has been associated with decrease in zinc concentration in RBC membrane resulting in reduced plasma sulphydryl concentration (Roth and Kirchgessner, 1991). Although, the present study did not measure erythrocyte zinc concentration, Kraus et al. (1997) have shown that increased oxidative demand leads to lowered erythrocyte zinc concentration. This may have accentuated the erythrocyte fragility in the CPF group. This is because zinc deficiency results in lower superoxide dismutase activity (Ruz et al., 1992) and a greater susceptibility to oxidative damage from elevated peroxynitrite concentrations (Powell, 2000). Similarly, lowered zinc level alters the composition of erythrocyte membranes (Avery and Bettger, 1992) and also impairs the activity and function of Na^+ K^+ ATPase, which is important for cell membrane stability (O'Dell et al., 1990). Furthermore, decrease in cellular zinc concentration has been associated with apoptosis induction via the protein kinase C-dependent pathways (Chou et al., 2004) and activation of apoptotic protease, caspase-3 (Duffy et al., 2001). Threfore, the amelioration of CPF-induced erythrocyte fragility by zinc may have partly resulted from its ability to inhibit caspase-3 (Perry et al., 1997) and the repletion of zinc deficit caused by oxidative stress (Chou et al., 2004).

In conclusion, the present study has shown for the first time the ability of zinc to ameliorate the increased erythrocyte fragility induced by chronic CPF exposure partly due to its antioxidant properties. Zinc may therefore be useful as a protective agent against CPF-induced toxic damage especially in individuals who are

occupationally challenged with daily low-dose CPF exposure.

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