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Review

Erythrocyte osmotic fragility and lipid peroxidation following chronic co-exposure of rats to chlorpyrifos and deltamethrin, and the beneficial effect of alpha-lipoic acid



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ABSTRACT

The present study aimed to evaluate the effect of chronic co-exposure to chlorpyrifos (CPF) and deltamethrin (DLT) on erythrocyte osmotic fragility, lipid peroxidation and the ameliorative effect of alpha-lipoic acid (ALA) on erythrocyte fragility. Thirty-six male Wistar rats divided into six groups of six rats each were used for the study. Groups I (S/oil) and II (ALA) were given soya oil (2 ml/kg) and ALA (60 mg/kg), respectively. Rats in group III (DLT) and IV (CPF) were exposed to DLT (6.25 mg/kg) and CPF (4.75 mg/kg) (1/20th of the previously determined LD₅₀ of 125 mg/kg and 95 mg/kg, respectively, over a period of 48 h). Rats in group V (CPF+DLT) were co-exposed to CPF (4.75 mg/kg) and DLT (6.25 mg/kg), while those in group VI (ALA+CPF+DLT) were pretreated with ALA (60 mg/kg) and then co-exposed to CPF and DLT, 45 min later. The treatments were administered by gavage once daily for a period of 16 weeks. Blood collected at the end of the experimental period were analyzed for erythrocyte osmotic fragility and malondialdehyde (MDA) concentration. The study showed that chronic co-exposure to CPF and DLT resulted in an increase in erythrocyte fragility and MDA concentration which were ameliorated by supplementation with alpha-lipoic acid. The study concluded that repeated co-exposure to CPF and DLT elevated erythrocyte fragility probably due to increased lipid peroxidation, and pretreatment with alpha-lipoic acid ameliorated these alterations.

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1. Introduction

Everyday humans and animals are exposed simultaneously and concurrently to a number of pesticides. Knowledge of adverse health effect following exposure to combination of pesticides is only limited even though the consumers are often exposed to more than one pesticide [1]. When pesticides are ingested, toxic effect may be observed, which differ quantitatively and/or qualitatively from those observed following exposure to the single pesticide [2]. Although the individual effects of pyrethroids and organophosphates (OPs) have been well documented, their combined toxicity has not been studied so far in detail. The wide spread use of pesticide combinations in agriculture and public health drew our attention to studying their possible toxic effects on erythrocytes in rats.

Chlorpyrifos (CPF) and deltamethrin (DLT) are OP and pyrethroid insecticides, respectively, that are widely used for a variety of agricultural and public health applications [3,4]. Although they have different main mechanism of toxicity, studies have shown that they can induce increase in oxidative damage in cells from various organs. The ability of OP and pyrethroid insecticides to induce oxidative stress in the erythrocyte membranes has been demonstrated [5–7]. The normal erythrocyte membrane is remarkable for its durability but its tolerance gets altered under certain pathological conditions [8], and this may lead to perturbation in the structural integrity of erythrocytes, resulting in oxidative haemolysis. Erythrocyte osmotic fragility (EOF) is said to be an indirect method of assessing oxidative stress [9] and toxicity of pesticides [10], as it gives information about the total status of red cell metabolism and membrane stability [8].

Alpha-lipoic acid (ALA) a natural short-chain fatty acid containing sulfhydryl groups is a potent antioxidant that retains protective function in both its reduced and oxidized forms [11]. It possesses free radical scavenging and antioxidant-regenerating abilities [12] that have been shown to be effective in several conditions in which oxidative stress have been implicated, including pesticide toxicity [13].

Although many studies have evaluated the role of oxidative stress and the ameliorative effect of antioxidant vitamins on pesticide-induced oxidative damage, there is paucity of information on oxidative stress induced by

chronic co-exposure to CPF and DLT insecticides on erythrocytes and the beneficial effect of ALA.

2. Materials and methods

2.1. Experimental animals

Young adult male Wistar rats weighing between 120 and 150 g used for this experiment were obtained from the laboratory animal house of the Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria. The animals were allowed to acclimatize for at least 2 weeks in the laboratory prior to the commencement of the experiment, and were given access to fed standard rat chow and water *ad libitum*. Thirty six Wistar rats were used for the experiment while twenty four were used for the median lethal dose (LD₅₀) determination of the two insecticides.

2.2. Chemical preparation

CPF 20% emulsifiable concentration (EC), marketed as Termiphos (Sabero Organics, Gujarat Limited, India) and DLT 1.25% EC marketed as Deltaforce® (Sabero Organics, Gujarat Limited, India) were prepared by reconstituting in soya oil (Grand Cereals and Oil Mills Ltd., Jos, Nigeria). Alpha-lipoic acid (600 mg/tablet) (marketed and distributed by General Nutrition Corporation, Pittsburgh, PA 15222) was obtained from a Pharmaceutical store in Abuja, Nigeria, and dissolved in soya oil, just prior to its daily administration.

2.3. Determination of LD₅₀ of chlorpyrifos and deltamethrin insecticides

The LD₅₀ of each insecticide was determined in Wistar rats using the Lorke [14] method in two phases. Twelve rats each were used for both insecticides and death was recorded over a 48 h period. The LD₅₀ for CPF and DLT were then determined based on dose-response (death) using the Lorke method.

2.4. Experimental protocol

The rats were weighed, and randomly divided into six groups of six rats each. Group I served as the control (S/oil) and was given soya oil only at a dose of 2 ml/kg, while group II (ALA) was dosed with alpha-lipoic acid only at a dose of 60 mg/kg [15]. Group III (DLT) was administered with DLT only at a dose of 6.25 mg/kg (corresponding to 1/20th LD₅₀ value: 125 mg/kg determined previously for this study), while group IV (CPF) was administered with CPF only at a dose of 4.75 mg/kg (corresponding to 1/20th LD₅₀ value: 95 mg/kg determined previously for this study). Rats in group V (CPF + DLT) were co-exposed to CPF (4.75 mg/kg) and DLT (6.25 mg/kg), while rats in group VI (ALA + CPF + DLT) were pretreated with ALA (60 mg/kg), and then co-exposed to CPF (4.75 mg/kg) and DLT (6.25 mg/kg), 45 min later. The different treatments were administered once daily by gavage for a period of 16 weeks. At the end of this study period, the rats were sacrificed by severing the jugular vein following light ether anesthesia. The study was carried out according to the specification of the Ahmadu Bello University Animal Research Committee and Guide to the Care and Use of Laboratory Animals [16].

2.5. Evaluation of erythrocyte osmotic fragility

1 ml of blood collected into heparinized sample bottles were analyzed for erythrocyte osmotic fragility using the method described by Faulkner and King [17] as modified by Oyewale [18]. Briefly, freshly obtained heparinized blood from each rat was pipetted into a set of test tubes containing 0.0, 0.1, 0.3, 0.5, 0.7, 0.9 g/L of NaCl (pH 7.4), and thereafter carefully mixed and incubated for 30 min at room temperature (26–28 °C). The test tubes were centrifuged at 800 × g for 10 min using a centrifuge model IEC HN-SII (Damon/IEC Division, UK). The supernatant was carefully transferred into a glass cuvette and the absorbance of the supernatant read colorimetrically using Spectronic 20 (Bausch and Lomb, USA) at a wavelength of 540 nm. The percentage haemolysis for each sample was calculated using the formula below by Faulkner and King [17]:

$$\text{percentage haemolysis} = \frac{\text{optical density of test solution}}{\text{optical density of standard solution}} \times 100$$

2.6. Evaluation of erythrocyte malondialdehyde concentration

Furthermore, 2 ml of heparinized blood samples obtained from each rat was centrifuged at 3000 × g and the plasma discarded. By washing erythrocytes three times in cold isotonic saline (0.9%, w/v), erythrocyte packets were prepared and used to assay for malondialdehyde (MDA) concentrations using the double heating method of Draper and Hadley [19] as modified by Yavuz et al. [20]. The principle of the method was based on spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with MDA. 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. The method of Dacie and Lewis [21] was used to evaluate the hemoglobin concentration in the washed erythrocytes.

2.7. Statistical analysis

Values obtained were expressed as mean ± SEM and were further subjected to one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, using GraphPad Prism version 5.0 for windows from GraphPad Software, San Diego, CA, USA (www.graphpad.com). Values of $P < 0.05$ were considered to be significant.

3. Results

3.1. LD₅₀ determination for CPF and DLT

The oral LD₅₀ of CPF and DLT in rats determined by the method of Lorke were 95 mg/kg and 125 mg/kg, respectively. The signs of toxicity appeared within two hours after dosing and they include excitation, salivation, lacrimation, sluggishness, watery diarrhea, huddling, intermittent convulsion and death for the CPF insecticide, and huddling, restlessness, tremors, writing convulsion and death for DLT insecticide.

3.2. Effect of treatments on *in vitro* erythrocyte osmotic fragility

There was complete haemolysis (100%) in the control solvent containing 0.0 g/L NaCl concentration (distilled water). There were no significant differences ($P > 0.05$) in the percentage haemolysis between the rats in the various treatment groups at 0.1, 0.3 and 0.7 g/L of NaCl. There were however, significant changes in the percentage erythrocyte fragility between the groups at 0.5 and 0.9 g/L of NaCl. At 0.5 g/L NaCl, there was a significant increase ($P < 0.05$) in the percentage erythrocyte fragility in the CPF + DLT group, compared to either the ALA or ALA + CPF + DLT group. Similarly, the percentage erythrocyte fragility in the CPF + DLT group was significantly higher ($P < 0.05$), compared to the ALA group at 0.9 g/L of NaCl concentration (Fig. 1). Percentage erythrocyte fragility was highest in the CPF + DLT group compared to other treatment groups at the various NaCl concentrations.

3.3. Effect on erythrocyte malondialdehyde concentration

The effect of treatments on erythrocyte MDA concentration is shown in Fig. 2. The MDA concentration was significantly higher ($P < 0.05$) in rats in the DLT, CPF, CPF + DLT and ALA + CPF + DLT groups, compared to the S/oil and ALA groups, respectively. The erythrocyte MDA concentration increased by 80.27%, 86.63% and 134.60%, in the DLT, CPF and CPF + DLT groups, respectively, compared to the S/oil group. The erythrocyte MDA concentration in the

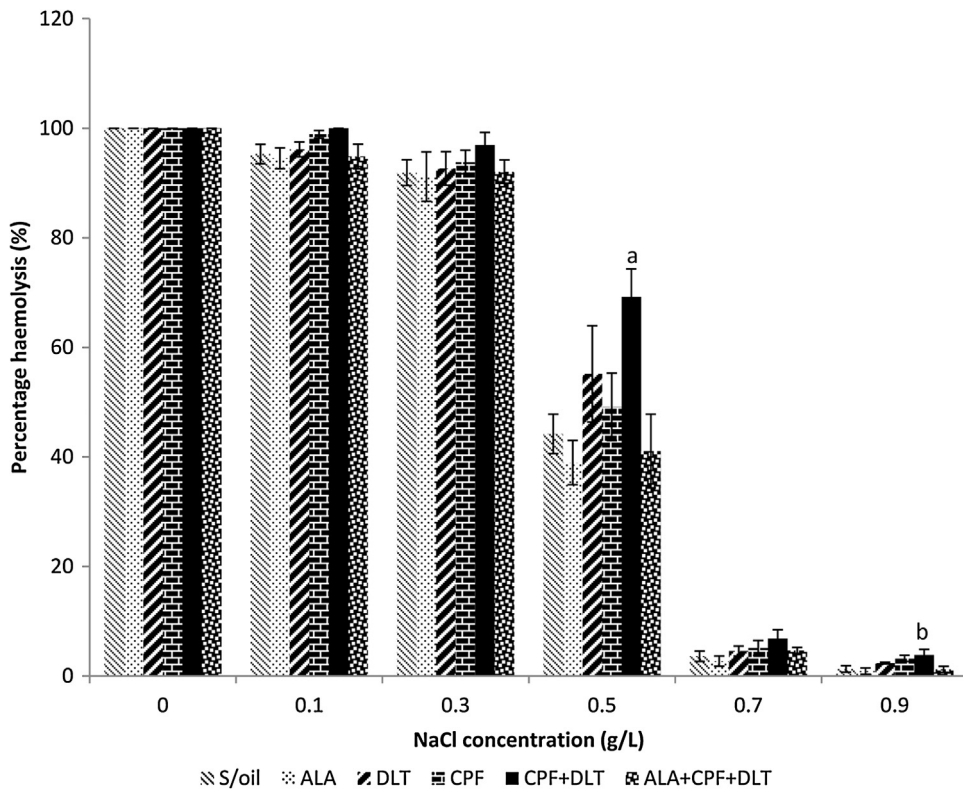


Fig. 1. Effect of treatments on erythrocyte osmotic fragility. ^aValues in chlorpyrifos + deltamethrin group significantly higher compared to ALA and ALA + chlorpyrifos + deltamethrin group ($P < 0.05$) at 0.5 g/L NaCl concentration. ^bValues at chlorpyrifos + deltamethrin group significantly higher compared to ALA group ($P < 0.05$) at 0.9 g/L NaCl concentration.

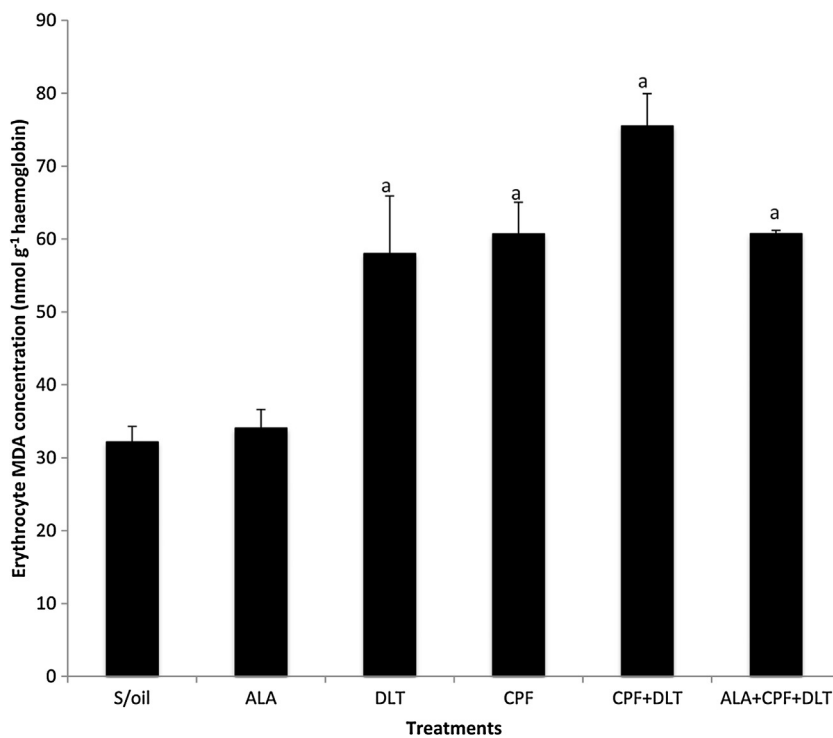


Fig. 2. Effect of treatments on erythrocyte malondialdehyde concentration. ^aValues in chlorpyrifos + deltamethrin, chlorpyrifos, deltamethrin and ALA + chlorpyrifos + deltamethrin groups, respectively, significantly higher compared to ALA and Soya oil groups ($P < 0.05$), respectively.

ALA+CPF+DLT group ($P>0.05$) decreased by 19.57% compared to the CPF+DLT group.

4. Discussion

The present study has clearly shown the ability of co-exposure to CPF and DLT to increase erythrocyte fragility due to increased lipid peroxidative damage to the erythrocyte membrane of rats as observed with the increase in erythrocyte MDA concentration. The increased erythrocyte MDA recorded in this study indicates that the pesticides promote the peroxidation of lipid membranes of the erythrocytes. Membrane lipids are vital for cellular integrity maintenance and survival [22]. Although erythrocytes are well equipped with several biological mechanisms to defend against intracellular oxidative stress [23], they can be oxidatively damaged due to toxic chemicals and environmental pollutants. MDA is a major oxidative product of peroxidized polyunsaturated fatty acids (PUFA), and elevated MDA concentration is an indicator of lipid peroxidation (LPO) [24]. Kale et al. [5] demonstrated that pesticides through LPO can disturb the biochemical and physiological functions of the red blood cells. The result recorded in the present study is consistent with reports that the OP, CPF [6,7,25] and pyrethroids, [26,27] individually induce an elevation in erythrocyte MDA concentration in exposed rats. The elevated erythrocyte MDA concentration was more pronounced in the co-exposed group compared to either the CPF or DLT alone group. This effect could be attributed to the increase in oxidative stress due to the combined effect of these lipophilic pesticides and other factors not captured in this study. The lipid peroxidative alteration in the structural and functional components of the erythrocyte membrane may have caused perturbations in the membrane integrity resulting in increased erythrocyte fragility recorded in the DLT+CPF group in the present study. The increased EOF observed indicates the ability of the pesticides to compromise the integrity of the erythrocyte membrane apparently from increased oxidative damage to the erythrocyte membrane [28] which may result in anemia, as anemia has been recorded by other workers following pesticides intoxication [29,30]. This anemia recorded by these authors may be sequel to the haemolysis caused by increased EOF by the pesticides. The increased EOF observed in the present study is consistent with those earlier reported on CPF intoxication in rats [7,31,32]. The aggregation of the pesticides on the lipid bilayer with a consequent increase in the membrane instability leading to osmotic fragility [33] may be the reason for the increased EOF in this study. The lipophilic nature of the two pesticides may have also enhanced lipid peroxidation by directly interacting with the cellular plasma membrane [34]. Free radicals generated by hemoglobin in the erythrocyte, which is a major source producing radicals on interaction with xenobiotics may have also led to increased erythrocyte membrane LPO and haemolysis [35]. EOF was highest in the co-exposed group in all the NaCl concentrations compared to either the CPF or DLT group, indicating a higher level of haemolysis in this group. The toxicity of CPF+DLT is more evident because it increased

EOF and elevated MDA concentration more than either the CPF or DLT exposed rats.

Supplementation with ALA as observed in the ALA+CPF+DLT group decreased the elevated erythrocyte MDA and osmotic fragility. This may be due to the antioxidant ability of ALA and its ability to regenerate other antioxidants such as vitamins E and C, and GSH from their radical or inactive forms. Caylak et al. [36] demonstrated the ability of ALA to offer protection to the erythrocytes against lead-induced oxidative stress in rat erythrocyte. The reduction of the EOF and MDA concentration by ALA observed in the ALA+CPF+DLT group further confirms the role of oxidative stress in the toxic mechanism of erythrocyte damage observed in the CPF+DLT intoxicated rats. The ability of ALA to preserve cellular membrane integrity through its capacity to exhibit membrane modulatory effects as reported by Arivazhagan et al. [37] may be responsible for this ameliorative effect.

In conclusion, the present study demonstrated the ability of chronic co-exposure to CPF and DLT in rats to increase erythrocyte fragility due to elevated lipid peroxidative changes in the erythrocyte membranes which may increase its vulnerability to lysis and anemia probably. The enhancement of erythrocyte LPO and increase in EOF may suggest involvement of oxidative stress in this pesticide combination toxicity. In addition, pretreatment with alpha-lipoic acid ameliorated the CPF+DLT-induced lipid peroxidative changes and subsequently reduced the erythrocyte fragility. The result obtained in the study may be relevant for the care of the health of people who are in direct or indirect contact with pesticides and their combinations.

Conflicts of interest

All the authors have no conflicts of interest.

Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

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