Comparative effects of Vitamin C and Acetyl-L-carnitine on subacute Chlorpyrifos-induced Erythrocyte Osmotic Fragility in Wistar rats

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ABSTRACT

Lipid peroxidation which can be measured indirectly by erythrocyte osmotic fragility is a molecular mechanism implicated in chlorpyrifos poisoning. The aim of the present study was to evaluate the comparative effects of vitamin C and acetyl-L-carnitine on subacute CPF-induced erythrocyte osmotic fragility in Wistar rats. Forty-nine adult male Wistar rats divided into 7 groups of 7 animals each were used as experimental subjects. Rats in groups I (S/oil), II (VC), III (ALC) and IV (CPF) were exposed to soya oil only (2 ml/kg), vitamin C (100 mg/kg), acetyl-L-carnitine (300 mg/kg) and chlorpyrifos only (8.5 mg/kg~ $1/10^{th}$ of the LD_{50}), respectively. Groups V (VC+CPF) and VI (ALC+CPF) were pretreated with vitamin C (100 mg/kg) and acetyl-L-carnitine (300 mg/kg), respectively, and then dosed with CPF (8.5 mg/kg) 30 min later. While group VII (VC+ALC+CPF) was pretreated with the combination of VC (100 mg/kg) and ALC (300 mg/kg) and then dosed with CPF (8.5 mg/kg), 30 min later. These regimens were administered orally once daily for a period of 28 days. At the end of the study, the rats were sacrificed and erythrocyte analyzed for erythrocyte osmotic fragility. The study showed that subacute CPF exposure caused increase in erythrocyte osmotic fragility, which was significantly decreased (P <0.05) by vitamin C, but differentially decreased (P >0.05) by ALC and the combination of VC and ALC. In conclusion, the study showed that CPF-evoked erythrocyte fragility was best ameliorated by pretreatment with vitamin C alone.

Key words: Chlorpyrifos, vitamin C, acetyl-L-carnitine, lipid peroxidation, erythrocyte membrane.

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INTRODUCTION

Organophosphate (OP) insecticides are chemicals which inhibit cholinesterase and are employed widely as pesticides in agricultural and residential settings [1]. As a result of their use which remain pervasive in both developed and developing nations, concerns are increasing regarding

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their relative safety to man, animals and the environment, as OP poisoning continues to be a cause of morbidity and mortality in third world countries [2].

One of such OP which has and is still spurring renewed interest is chlorpyrifos (CPF). Chlorpyrifos is a broad spectrum chlorinated OP insecticide used heavily through out the world for agricultural and domestic pest control. Anaemia has been observed following chronic [3], acute [4] and subacute [5] exposure to CPF in rats. Although, the molecular mechanism of anaemia has not been fully elucidated, studies have shown that CPF results in lipoperoxidative changes in erythrocyte membrane [6-7]. Oxidative stress which results from the generation of free radicals and alteration in free radical scavenging system is one of the complex factors that determine the integrity of the erythrocyte [8]. Erythrocyte osmotic fragility is frequently used as a measure of the strength of the red blood cells [9]. Earlier studies have shown that vitamin C [10] and acetyl-L-carnitine [5] ameliorated increased in erythrocyte fragility following chronic and subacute CPF exposure.

Vitamin C (VC) is a water soluble antioxidant that is reported to neutralize reactive oxygen species (ROS) and reduce oxidative damage [11], while acetyl-L-carnitine (ALC) is a vital co-factor for the mitochondrial oxidation of fatty acid [12], and is known to prevent the formation of ROS, scavenge free radicals and protect cells from peroxidative stress [13]. The study was aimed at comparing the individual effect and/or synergistic effect of vitamin C and acetyl-L-carnitine in attenuating subacute CPF-induced erythrocyte osmotic fragility in Wistar rats.

MATERIALS AND METHODS

Forty-nine young adult male Wistar rats (10-12 weeks old) weighing 120-140g used for this study were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The animals were housed in metal cages and fed on standard rat pellets, with water provided *ad libitum*. The experiment was carried out in accordance with the Guide for the Care and Use of Laboratory Animals [14].

Chemicals
Commercial grade CPF, TERMICOT[®] (20% EC, Sabero Organics, Gujarat Limited, India), was reconstituted in soya oil (10%) prior to daily administration. ALC (500 mg/capsule); L-carnipure[®] (Ideasphere Inc. America Fork UT84003 U.S.A) was reconstituted also in soya oil prior to daily administration. Vitamin C tablets (Mopson Pharmaceutical Ltd, Lagos, Nigeria) were prepared in distilled water to make 10% stock solution.

Animal Treatment Schedule
The rats were weighed and then divided at random into 7 groups of 7 animals in each group. Group I (S/oil) was given only soya oil (2 mlkg^{-1}). Groups II (VC) and III (ALC) were administered vitamin C (100 mgkg^{-1}) and ALC (300 mgkg^{-1}), respectively. While group IV (CPF) was dosed with CPF only [8.5 mgkg^{-1}, ~ 1/10th LD$_{50}$ as determined by Uchendu (2011)]. Groups V (VC + CPF) and VI (ALC+CPF) were pretreated with VC (100 mgkg^{-1}) and ALC (300 mgkg^{-1}), respectively, and then dosed with CPF (8.5 mgkg^{-1}), 30 min later. Rats in group VII (VC+ALC+CPF) were pretreated with the combination of VC (100 mgkg-1) and ALC (300 mgkg-1), and then dosed with CPF (8.5 mgkg^{-1}), 30 min later. The regimens were administered

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once daily by gavage for a period of 28 weeks. At the end of the treatment period, the rats were sacrificed via jugular venesection after light chloroform anaesthesia. 2 ml of blood sample was collected from each animal into EDTA bottles, and were used to analyze for erythrocyte osmotic fragility.

Evaluation of Erythrocyte Osmotic Fragility
In vitro erythrocyte osmotic fragility was evaluated in all the rats in each group using the method described by Fraulkner and King [16] as modified by Oyewale [17], using different amounts of sodium chloride (pH 7.4) from 0.0, 0.1, 0.3, 0.5, 0.7 and 0.9 g/L of distilled water. Briefly, freshly obtained whole blood from each rat was pipetted into the test tubes containing varying concentration of NaCl and then followed by careful, gentle mixing and incubation for 30 minutes at room temperature, 26-28°C. The samples were then centrifuged at 600 g for 10 minutes using a centrifuge model IEC HN-SII (Damon IJEC 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 a wave length of 540 nm. The percent haemolysis for each sample was calculated based on the formula;

$$\text{Percent haemolysis} = \frac{\text{Optical density of test solution}}{\text{Optical density of standard solution}} \times 100$$

Statistical Analysis
Values obtained were expressed as Mean ± SEM and subjected to one way analysis of variance followed by Tukey's multiple comparison test using Graph Pad Prism 4.0. Values of $P < 0.05$ were considered significant. Similarly, the mean differences between the groups were expressed as percentages when $P > 0.05$.

RESULT

Effect on *in vitro* erythrocyte osmotic fragility
Generally, irrespective of the treatment groups, percentage haemolysis decreased with increasing NaCl concentration. Haemolysis was complete (100%) in the standard solvent (distilled water). There were no significant changes ($P > 0.05$) in the degree of erythrocyte fragility among the rats in the various groups at 0.1, 0.3, 0.5 and 0.7% of NaCl concentration. Although, the CPF group showed the highest mean percentage haemolysis which decreased considerably in the VC+CPF, ALC+CPF and VC+ALC+CPF groups, respectively. There was a significant decrease ($P < 0.05$) in erythrocyte osmotic fragility at 0.9% NaCl in the VC+CPF group when compared to the CPF group, as no haemolysis was observed in the VC+CPF group (Figure 1).
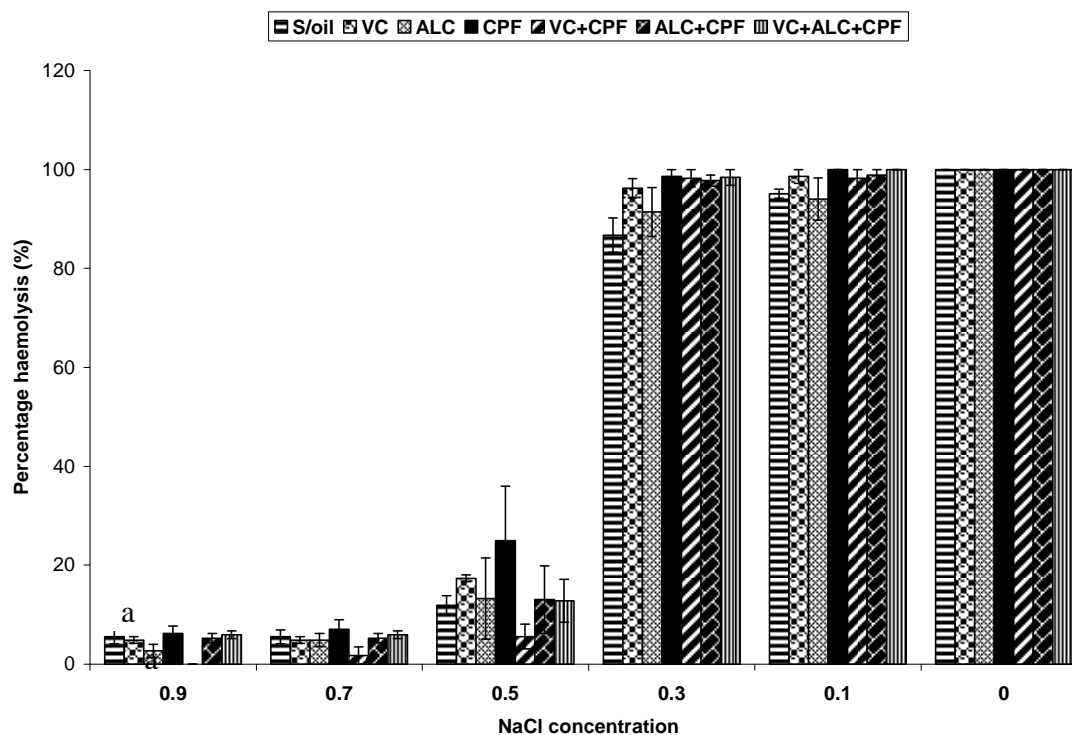
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Figure 1: Effect subacute administration of soya oil, Vitamin C, acetyl-L-carnitine and chlorpyrifos on erythrocyte osmotic fragility in Wistar rats.
Superscript with the same alphabet are significant ($^aP < 0.05$)

DISCUSSION

The erythrocyte osmotic fragility, which can be used as an indirect method of evaluating lipid peroxidation in animals [18] has been shown to increase in the present study following repeated CPF exposure in Wistar rats compared to the other groups. This may be due to the ability of CPF to compromise the integrity of RBC membrane apparently from increased oxidative damage to erythrocyte membrane [19]. Significant increase in erythrocyte osmotic fragility has been reported following CPF exposure [7, 10, 20]. The structural integrity of the erythrocyte membrane is an important feature for its resistance to peroxidative attack. However, erythrocytes are susceptible to oxidative stress due to large amount of unsaturated membrane phospholipids [21]. Process of lipid peroxidation decreases hydrophobic characteristic of bilayer membrane of erythrocytes, altering affinity and interaction of protein and lipid thereby impairing the functioning and homoestasis of erythrocyte membrane [22]. Lipid peroxidation, which is the process of oxidative degradation of polyunsaturated fatty acids when it occurs in biological membrane as shown in the CPF group, causes impairment of membrane function and structural conformation [23].

The significant decrease in erythrocyte osmotic fragility by the antioxidant vitamin C demonstrates the role of oxidative stress in the toxic mechanism of CPF exposure. In fact, vitamin C completely protected against haemolysis of the erythrocyte of rats exposed to CPF at 0.9% NaCl concentration in the present study. This agreed with the findings of [10]. Ambali *et al.* [24] also demonstrated the mitigative effect of vitamin C on erythrocyte lipoperoxidative

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changes induced by co-administration of CPF and lead. Devi *et al*. [25] reported that vitamin C supplementation decreased oxidative damage to erythrocytes. Vitamin C is known to protect completely lipids in plasma and low density lipoprotein against peroxidative damage [26].

The improvement in erythrocyte osmotic fragility seen in the ALC+CPF group may be due to the ability of ALC to aid the maintenance of red blood cells membrane stability [27] and also decrease osmotic fragility [28]. This finding agreed with that observed by [5]. This also further confirmed the role of lipid peroxidation in the toxic mechanism of erythrocyte damage following subacute CPF exposure. Furthermore, pretreatment with vitamin C was able to decrease erythrocyte osmotic fragility induced by CPF at 0.3%, 0.5% and 0.7% NaCl concentration by 0.4%, 77.7% and 75%, respectively, in comparison with ALC which was able to decrease erythrocyte osmotic fragility at the same NaCl concentration by 0.88%, 47.7% and 25.3%, respectively.

From the present study, it seems paradoxical that vitamin C showed stronger protective effect on the erythrocyte than the combination of VC and ALC showing that pretreatment with vitamin C and ALC did not produce significant enhanced antioxidant effect. This can be explained by the diversity of the mechanisms by which antioxidant restrict lipid peroxidation by free radicals. Denisov and Azatyan [29] explained that co-administration of two inhibitors of free radicals to an oxidized hydrocarbon or other substance may exhibit a net additive, synergistic or antagonistic effect. It was not unlikely therefore that a net synergistic effect was the outcome following co-administration of vitamin C and ALC. The strongest effect shown in the present study on erythrocyte osmotic fragility as a result of supplementation with vitamin C alone agreed with the observation by Kraus *et al*. [30] who demonstrated the effect of vitamins C, E or β-carotene on osmotic fragility of zinc deficient rats.

In conclusion, the present study has further validated the ability of subacute CPF exposure to increase erythrocyte fragility. In addition, pretreatment with VC and/or ALC differentially ameliorated the increased erythrocyte fragility induced by subacute CPF partly due to their antioxidant properties, although VC exerted a stronger protective effect than VC and the combination of ALC and VC.

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