Protective Effects of Acetyl-L-Carnitine on Subacute Chlorpyrifos-induced Biochemical Changes in Wistar Rats


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ABSTRACT

Induction of oxidative stress is one of the molecular mechanisms involved in chlorpyrifos-induced toxicity. The study was aimed at evaluating the effect of Acetyl-L-Carnitine (ALC), an antioxidant molecule on alterations in biochemical changes evoked by subacute chlorpyrifos (CPF) exposure in Wistar rats. Twenty-eight young adult male Wistar rats used for the study were divided into 4 groups of 7 animals each. Group I was administered S/0il (2 mL kg⁻¹) while group II was given ALC (300 mg kg⁻¹). Group III was administered CPF (8.5 mg kg⁻¹) while group IV was pretreated with ALC (300 mg kg⁻¹) and then administered CPF (8.5 mg kg⁻¹), 30 min later. The regimen were administered orally via gavage for 4 weeks. The sera obtained from the blood samples were analysed for concentrations of electrolytes (Na⁺, K⁺, Cl⁻ and HCO₃⁻), total proteins, albumin, globulin glucose, urea, creatinine and activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and creatine kinase. The liver was also examined for malonaldehyde concentration and activities of superoxide dismutase and catalase. The result shows that ALC pretreatments attenuate CPF-evoked alterations in biochemical parameters apparently due to its antioxidant properties.

Key words: Organophosphates, chlorpyrifos, biochemical parameters, oxidative stress, acetyl-L-carnitine

INTRODUCTION

Chlorpyrifos (CPF), a chlorinated organophosphate (OP) insecticide (Aly et al., 2010) is one of the most extensively used insecticides globally (Levin et al., 2001) in agriculture and public health (Al-Badrany and Mohammad, 2007). The widespread use of CPF raises the likelihood of inadvertent exposure to the pesticide in segments of the population (Cohn and Macphail, 1997), as exposure to human population continues to be nearly ubiquitous (Casida and Quistad, 2004).

Like other OP insecticides, the mechanism of CPF toxicity is related to irreversible acetylcholinesterase (AChE) inhibition resulting in cholinergic manifestations (Ambali et al., 2010b). However, evidence has shown that other non-cholinergic mechanisms may be implicated in its toxicity (Slotkin, 2004; Slotkin et al., 2006), since toxicity results at doses that does not inhibit AChE (Chakraborti et al., 1999). The induction of oxidative stress which is associated with enhanced production of Reactive Oxygen Species (ROS) leading to cellular damage is increasingly been linked to CPF toxicity (Gultekin et al., 2006; Ambali et al., 2007, 2010a). Assessment of biochemical parameters is an important tool in assessing the health status of the individuals and is valuable in predicting clinical and prognostic outcomes (Krishna and Ramachandran, 2009).
Alterations in biochemical profiles have been demonstrated in CPP toxicity in many studies (Goel et al., 2005; Ambali et al., 2007, 2010a). Similarly, the mitigating effect of some antioxidant on CPP-evoked oxidative damage has also been demonstrated by several workers (Goel et al., 2005; Ambali et al., 2007, 2010a; El-Hossary et al., 2009; Mansour and Mossa, 2010).

Acetyl-L-Carnitine (ALC) which is an ester of trimethylated amino acid, L-carnitine and synthesized in the brain, liver and kidney (Calabrese et al., 2005) is known to prevent the formation of ROS, scavenge free radicals and protects cells from peroxidative stress (Arockia Rani and Panneerselvam, 2001; Dokmeci et al., 2005, 2006). The present study therefore evaluated the effect of ALC in ameliorating alterations in biochemical parameters induced by subacute CPP exposure in Wistar rats.

MATERIALS AND METHODS

Twenty eight young adult male Wistar rats (10-12 weeks old) weighing 120-150 g 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.

Chemicals: Commercial grade CPP, 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 USA) was reconstituted also in soya oil prior to daily administration.

Animal treatment schedule: The rats were weighed and then divided at random into 4 groups of 7 animals in each group. Group I (S/oil) was given only soya oil (2 mL kg⁻¹). Group II (ALC) was administered ALC only (300 mg kg⁻¹) while group III (CPP) were dosed with CPP only 8.5 mg kg⁻¹, ~1/10th LD₅₀ as determined by Ambali (2009). Group IV (ALC+CPP) was pretreated with ALC (300 mg kg⁻¹) and then dosed with CPP (8.5 mg kg⁻¹), 30 min later. The regimens were administered once daily by gavage for a period of 4 weeks. At the end of the treatment period, the rats were sacrificed by severing the jugular vein after light chloroform anaesthesia and blood samples collected into test tubes, incubated for 30 min and then centrifuged at 800 xg for 10 min. The sera obtained were collected into clean test tubes and used for the evaluation of biochemical parameters. The study was carried out according to the specification of the Ahmadu Bello University Animal Research Committee and in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Serum biochemical analysis: The sera samples were evaluated for the concentrations of glucose, Total Proteins (TP), albumin, electrolytes (Na⁺, K⁺, Cl⁻ and HCO₃⁻), urea, creatinine and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Creatinine Kinase (CK) using an autoanalyzer (Bayer Clinical Chemistry Analyzer, Germany). Globulin concentration was obtained by subtracting the albumin concentration from that of the total protein.

Evaluation of hepatic lipoperoxidation: The malonaldehyde (MDA) concentrations in the liver samples as an index of lipid peroxidation evaluated using the double heating method of
Draper and Hadley (1990) as modified by Yavuz et al. (2004). The MDA concentrations were expressed as nmol g⁻¹ tissue protein.

**Evaluation of superoxide dismutase activity:** The hepatic superoxide dismutase (SOD) activity was evaluated using the method of Misra and Fridovich (1972). The SOD activity was expressed as units mg⁻¹ protein.

**Evaluation of catalase activity:** The catalase (CAT) activity was evaluated. The CAT activity was expressed as units mg⁻¹ protein.

**Determination of hepatic protein concentrations:** The hepatic protein concentration was determined according to the method of Lowry et al. (1951).

**Statistical analysis:** Data 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. Similarly, the mean differences between the groups were expressed as percentages when p>0.05.

**RESULTS**

**Effect of treatments on serum electrolytes concentration:** There was no significant (p>0.05) change in the serum sodium ion concentration in between the groups. However, the Na⁺ concentration in the CPF group decreased marginally compared to the Soil group (0.5%) and ALC+CPF (2.4%) groups.

There was no significant (p>0.05) change in the serum K⁺ concentration in between the groups. However, the K⁺ concentration slightly increased in the CPF group by 0.6 and 1.1% when respectively compared to the Soil and ALC+CPF groups (Fig. 1).

The serum Cl⁻ concentration showed no significant (p>0.05) change between the groups. However, the lowest mean Cl⁻ concentration was observed in the CPF group as it marginally decreased by 2 and 3% compared to the Soil and ALC+CPF groups, respectively (Fig. 1).

![Fig. 1: Effect of subacute administration of soya oil (Soil), Acetyl-L-Carnitine (ALC) and/or chlorpyrifos (CPF) on serum electrolytes concentration in Wistar rats](image_url)

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Fig 2: Effect of subacute administration of soya oil (S/oil), Acetyl-L-Carnitine (ALC) and/or chlorpyrifos (CPF) on serum glucose concentration in Wistar rats. Values with the same alphabets are significantly different

Fig 3: Effect of subacute administration of soya oil (S/oil), Acetyl-L-Carnitine (ALC) and/or chlorpyrifos (CPF) on serum total protein, albumin and globulin concentrations in Wistar rats. Values with the same alphabets are significantly different

No significant (p>0.05) difference was observed between the groups. The lowest mean value of HCO$_3^-$ concentration was observed in the CPF group as it decreased by 2.6 and 4% compared to the S/oil and ALC+CPF groups, respectively (Fig. 1).

**Effect of treatments on serum glucose concentration:** A significant reduction (p<0.01) in serum glucose concentration was observed in the CPF group when compared to the S/oil or ALC group. A significant decrease (p<0.05) in the glucose concentration was recorded in the ALC + CPF group when compared to the S/oil or ALC group. Although not significant, the mean glucose concentration in the ALC+CPF group increased by 32% when compared to the CPF group (Fig. 2).

**Effect of treatments on serum protein concentration:** There was no significant (p>0.05) change in the total protein concentration between the CPF and the S/oil groups. The TP level significantly (p<0.01) increased in the ALC+CPF group when compared to either S/oil or CPF group (Fig. 3).
There was no significant (p>0.05) difference in the serum albumin concentration between the treatment groups. However, the lowest serum albumin level was observed in the CPF group as it was marginally lower compared to the S/oi (14%), ALC (12.8%) and ALC+CPF (12%) groups (Fig. 3).

There was no significant (p>0.05) change in the globulin concentration in the CPF group compared to the S/oi or ALC group. A significant (p<0.05) increase in globulin concentration was recorded ALC+CPF group compared to the S/oi group. Although not significant, the globulin concentration in the ALC+CPF group increased by 49% relative to CPF group (Fig. 3).

There was no significant (p>0.05) change in the albumin/globulin (A/G) ratio between the groups (Fig. 4). However, the A/G ratio in the S/oi group decreased relative to ALC (56.5%), CPF (41.4%) and ALC+CPF (55%) groups.

**Effect of treatments on serum urea concentration:** The serum urea concentration in the CPF group was significantly (p<0.01) higher compared to ALC group. Although not significant (p>0.05), the urea concentration in the CPF group was comparatively higher compared to S/oi (20%) or ALC+CPF (7%) group. The urea concentration in the ALC+CPF group increased significantly (p<0.05) when compared to ALC group (Fig. 5).

**Effect of treatments on serum creatinine concentration:** The serum creatinine concentration showed no significant (p>0.05) difference between the groups. The highest mean value was recorded in the CPF group as it increased by 22, 13 and 8%, respectively, compared to the S/oi, ALC and ALC+CPF groups (Fig. 6).

**Effect of treatments on hepatic enzymes activity:** The effect of treatment on hepatic enzymes activities is shown in Fig. 7. The serum AST activity showed no significant (p>0.05) difference between the groups. The CPF group had the lowest AST activity as it relatively decreased compared to the S/oi (20.4%), ALC (4.2%) and ALC+CPF (24%) groups.

There was no significant (p>0.05) change in the ALT activity between the groups. However, the ALT activity was highest in the CPF group as it slightly increased by 7, 23 and 11% when compared, respectively to the S/oi, ALC and ALC+CPF groups.
Fig. 5: Effect of subacute administration of soya oil, Acetyl-L-Carnitine (ALC) and/or chlorpyrifos (CPF) on serum urea concentration in Wistar rats. Values of the same alphabets are significantly different.

Fig. 6: Effect of subacute administration of soya oil (S/oil), Acetyl-L-Carnitine (ALC) and/or chlorpyrifos (CPF) on serum creatinine concentration in Wistar rats.

Fig. 7: Effect of subacute administration of soya oil (S/oil), Acetyl-L-Carnitine (ALC) and/or chlorpyrifos (CPF) on liver enzyme activities in Wistar rats. Values with the same alphabets are significantly different.
Fig. 8: Effect of subacute administration of soya oil (S/oil), Acetyl-L-Carnitine (ALC) and/or chlorpyrifos (CPF) on creatine kinase activities in Wistar rats.

Fig. 9: Effect of subacute administration of soya oil (S/oil), Acetyl-L-Carnitine (ALC) and/or chlorpyrifos (CPF) on hepatic malonaldehyde concentration in Wistar rats. Values of the same alphabets are significantly different.

The ALP activity in the CPF group showed a significant (p<0.05) increase when compared to the S/oil group. Significant (p<0.05) increase in the ALP activity was also observed in the ALC group compared to the S/oil group. There was no significant change in the ALP activity in the ALC+CPF group compared to the CPF group, as it decreased by 28%.

**Effect of treatments on creatine kinase activity:** There were no significant (p>0.05) changes observed in CK activities between the groups. However, the highest CK activity was observed in the CPF group as it marginally increased compared to S/oil (13%), ALC (43.3%) and ALC+CPF (6%) groups (Fig. 8).

**Effect of treatments on hepatic malonaldehyde concentration:** A significant increase in hepatic MDA concentration was recorded in the CPF group compared to the S/oil (p<0.01), ALC (p<0.01) or ALC+CPF (p<0.05) group. There was no significant (p<0.05) change in the hepatic MDA concentration in the ALC+CPF group compared to the S/oil or ALC group (Fig. 9).
Fig. 10: Effect of subacute administration of soya oil (S/oil), Acetyl-L-Carnitine (ALC) and/or chlorpyrifos on hepatic superoxide dismutase activity in Wistar rats

Fig. 11: Effect of subacute administration of soya oil (S/oil), Acetyl-L-Carnitine (ALC) and/or chlorpyrifos on hepatic catalase activity in Wistar rats

**Effect of treatment on catalase and dismutase activity:** Effect of treatment on Catalase and Dismutase activity is shown in Fig. 10 and 11, respectively.

**DISCUSSION**

Generally, the present study did not record significant change in many of the biochemical parameters evaluated in the CPF group. There is however some level of alterations in these parameters which in clinical settings gives some room for concern. This is important considering the fact that low dose CPF mimicking environmental exposures were used. The alterations in many of the biochemical parameters evaluated in the present study indicate that some level of tissue pathological changes did occur following exposure to this low dose of insecticide.

Although, the present study did not record a significant change in serum electrolyte concentrations, the fact that there were some level of alterations should not be totally ignored as it demonstrates this low level CPF exposure may cause some level of metabolic changes. Ambali et al. (2007) recorded a non significant change in Cl⁻ and K⁺ concentrations but showed a significant elevation in Na⁺ concentration apparently due to diarrhoea recorded. However, the marginal decrease in the serum Cl⁻ concentration recorded in the CPF group when compared to the
other groups in this study may be associated with functional alteration in the proximal tubules of the nephron (Krishna and Ramachandran, 2009), but was reversed following pretreatment with ALC. The improvement in Cl− concentration in ALC+CPF group is suggestive of their protective effect on CPF-induced renal lesion apparently due to their antioxidant effect.

The present study also demonstrated that subacute CPF exposure caused a significant decrease in serum glucose level. This finding was in agreement with those recorded in previous studies (Szabo et al., 1988; Zama et al., 2005; Krishnamoorthy et al., 2006; Akhtar et al., 2009). This, however, contradicted the hyperglycaemia previously reported by Ambali (2009). The reason for the hypoglycaemia may be due to the impairment in hepatic gluconeogenesis due to CPF-evoked hepatotoxicity which has been demonstrated in the present study and previous ones (Goel et al., 2005; Ambali et al., 2007). The improvement in glucose concentration in the ALC+CPF group may be due to attenuation of CPF-evoked oxidative stress by ALC. This may have been responsible for the restoration of the serum glucose concentration. In addition, the ability of ALC to increase the level of amino acid in the liver may have provided additional ingredients for hepatic gluconeogenesis (Calabrese et al., 2006).

The study also showed an insignificant reduction in the concentration of serum TP in rats subacutely exposed to low-dose CPF. Significant reduction in serum TP have been reported following CPF exposure in previous studies (Khan and Kour, 2007; Akhtar et al., 2009; Obaineh and Mathew, 2009; Ambali, 2009). Although there was no significant change in the serum protein concentration in the CPF group, the study did record a relatively lower albumin concentration in the group. This apparent reduction, although not significant, did suggest some level of hepatocellular injury which has been demonstrated following CPF exposure in previous studies (Goel et al., 2005; Ambali et al., 2007, 2010c; Ambali, 2009). The relatively lower albumin concentration in the CPF group may also be due to its OP-scavenging properties (Peeples et al., 2005). Furthermore, the use of albumin as an antioxidant (Roche et al., 1999) in scavenging for CPF-evoked reactive oxygen species may have contributed to its apparent reduction in the CPF group.

The relative increase in serum globulin concentration in the CPF group agreed with that observed by Subbotina and Belonozhko (1968) following exposure to an OP compound, sevin. The result, however, contradicted those obtained by Szabo et al. (1988) and Ambali (2009) following repeated CPF exposure in rats. OP exposures have been reported to induce the formation of antibodies to the nervous tissues (McConnell et al., 1999) which may have been responsible for the increased globulin observed in the CPF group in the present study. The apparent decrease in the albumin/globulin ratio in the CPF group further confirms the relative increase in globulin concentration recorded in this group.

ALC supplementation has been shown in the present study to improve the total protein and albumin concentrations but caused a further increase in globulin concentration as confirmed by a further decrease in the albumin/globulin ratio. The improvement in the total proteins and albumin concentrations may be partly due to its antioxidant properties which protects the liver from CPF-evoked oxidative damage. The further increase in globulin concentration in group supplemented with ALC may be due to immunostimulatory effect of ALC.

Although, the serum urea concentration in the CPF group did not differ significantly compared to the Skoil group, 20% increase in the former is a cause for concern as it shows some level of renal pathological changes (Ambali et al., 2007; Kerem et al., 2007; Ambali, 2009). This renal change which agrees with previous findings (Krishnamoorthy et al., 2006; Ambali et al., 2007, 2010c) may
have been due to oxidative damage. However, pretreatment with ALC resulted in slight decrease in urea concentration indicating its tendency to protect the renal tissue from CPF-evoked lesion. This may be partly due to its antioxidant effect which protected the renal tissue from lipoperoxidative changes.

The relative increase in creatinine concentration in the CPF group is an indication of some form of pathological changes in the muscle and/or kidneys. Creatinine, a by product of muscle metabolism (Quintanilla, 1982) is ordinarily been excreted in the urine by the kidneys. However, in renal insufficiency or obstruction, an elevated creatine concentration results (Ambali et al., 2007). Previous studies have shown the ability of CPF to increase serum creatinine concentration (Krishnamoorthy et al., 2006; Ambali et al., 2007, 2010c; Ambali, 2009). Pretreatment with ALC restored creatinine concentration to almost a normal level due to its protective role against OP-induced renal and muscle damages induced by CPF, probably due to its antioxidant effect.

Subacute CPF exposure was shown in this study to cause a non-significant decrease in AST activity. Although, the cause and toxicological significance of low AST activity is not known, similar results have been recorded previously (Barne-Lloyd et al., 1991; Ambali et al., 2007). However, this finding contradicts those of other workers (Goel et al., 2005; Zama et al., 2005; Khan and Kour, 2007; Ambali, 2009). It is possible that CPF directly inhibited AST activity, similar to what was observed with ALT by Altuntas and Delibas (2002). The relative increase in serum ALT activity recorded in the CPF group was in consonant with the findings from earlier studies (Goel et al., 2005; Zama et al., 2005; Khan and Kour, 2007; Ambali, 2009). ALT a highly liver specific enzyme (Lukaszewicz-Hussain and Moniuszko-Jakoniuk, 2005) is one of the most reliable indicator of hepatotoxic damage (Ozer et al., 2008). ALT is primarily localized to liver, with lower enzyme activities found in skeletal muscle and heart tissue (Ozer et al., 2008). The apparent increase in ALT activity observed in rats exposed to CPF only in the present study may be due to increased hepatic lipid peroxidation that has been reported in the present study and other earlier ones (Ambali et al., 2010a; Ambali, 2009). The significant increase in serum ALP activity in rats exposed to CPF only agreed with previous works (Goel et al., 2005; Ambali et al., 2007; Khan and Kour, 2007; Ambali, 2009). This shows that subacute CPF exposure caused pathological lesions in any or all the organs involved in the synthesis and/or release of ALP such as the kidneys, bones, muscles, intestinal mucosa and especially the liver, probably due to oxidative damage. Paradoxically, however, the apparent increase in ALT and ALP activities recorded in the ALC group is pointing to hepatic damage. The reason for this is not known and will require further study.

Pretreatment with ALC was able to restore the activities of these enzymes to almost the level recorded in the S0 group. This may be due to the antioxidant property of ALC that protected the hepatocytes from CPF-induced oxidative damage.

Chlorpyrifos caused a slight elevation in Creatine Kinase (CK) activity, indicating muscular damage (Friedman et al., 2003). This finding agreed with that of Zama et al. (2005). Elevated CK activity and rhabdomyonecrosis of the skeletal muscle have been reported following OP exposure (Friedman et al., 2003; Lau et al., 2003). Pretreatment with ALC was able to restore the CK activity to near normal level, probably due to its antioxidant properties which may have prevented oxidative muscular damage.

The elevated hepatic MDA concentration in the CPF group indicates increased lipoperoxidation. This finding was in agreement with that reported by other workers (Goel et al., 2005; Zama et al., 2007; Ambali, 2009). The levels of MDA, a major product of peroxidation of polyunsaturated fatty
acids have been considered as an important indicator of lipid peroxidation (Kalender et al., 2004; Aly et al., 2010). Thus, the increase MDA concentration could be due to increase in pesticide-induced ROS generation and inhibition of antioxidant enzymes function (Gultekin et al., 2001). The increase in hepatic lipid peroxidation following repeated exposure to CPF may have caused membrane damage resulting in considerable ultrastructural damage to the liver cells and eventually loss of membrane integrity (Khan and Kour, 2007). Lipid peroxidation has been suggested as one of the non-cholinergic mechanisms involved in pesticide-induced toxicity (Kohrer, 1993; Ambali et al., 2007). Pretreatment with ALC on the other hand, reduced the liver MDA concentrations, indicating amelioration of CPF-evoked lipoperoxidation, apparently due to its antioxidant properties.

The apparent inhibition of hepatic SOD and CAT activities in the CPF group observed in this study is an indication that exposure to CPF modifies the endogeneous antioxidant enzymes with increased tendency towards oxidative stress. This was consistent with the findings recorded in previous studies (Gultekin et al., 2000; Zama et al., 2007; Aly et al., 2010). A reduction in SOD activity favours the accumulation of oxygen free radicals in the liver leading to tissue damage as a result of oxidative binding of key intracellular molecules containing thiol groups (Lopez et al., 2007). Lower CAT activity may have resulted from increased superoxide radical production arising from decreased SOD activity (Zama et al., 2007). Kono and Fridovich (1982) showed that superoxide radicals can directly inhibit the CAT activity. Pretreatment with ALC increased hepatic SOD and CAT activities apparently due to decreased lipoperoxidation recorded in this group. Increase in hepatic SOD and CAT activities accelerated the removal of ROS therefore reducing hepatic injury.

CONCLUSION
The present study has shown that repeated low level exposure to CPF resulted in alteration of biochemical profiles of rats depicting some levels of pathological changes in the liver, kidney and muscles. Lipoperoxidation as exemplified by increased MDA concentration and decreased activities antioxidant enzymes in the liver of rats exposed to CPF may be partly responsible for this organ toxicity. Pretreatment with ALC has been shown by the present study to ameliorate the CPF-evoked biochemical alterations partly due to its antioxidant properties which protects tissue from lipoperoxidation.

REFERENCES


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